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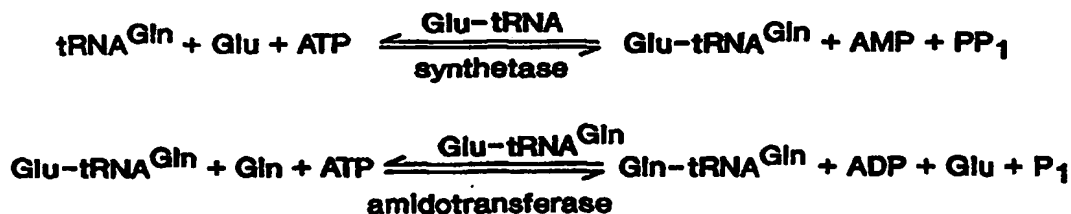


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(54) Title: GLUTRNA^{Gln} AMIDOTRANSFERASE - A NOVEL ESSENTIAL TRANSLATIONAL COMPONENT



(57) Abstract

The present method provides the amino acid sequence and encoding nucleic acid sequence of GlutRNA^{Gln} amidotransferase (AdT), a protein that is essential for protein translation. The AdT proteins and encoding nucleic acid molecules herein described can be used as targets for identifying agents that block translations. Such agents can be used as an antimicrobial, antifungal or herbicide agent.

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GlutRNA^{Gln} Amidotransferase - A Novel Essential Translational Component

CROSS REFERENCE TO RELATED APPLICATIONS

This application is related to U.S. Provisional Application Serial No. 60/037,275; filed February 3, 1997, which is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention is in the field of inhibitors of protein translation, particularly translation of proteins within microorganisms and organelles. This invention relates to newly identified polynucleotides and polypeptides, and their production and uses, as well as their variants, agonists and antagonists, and their uses. In particular, in these and in other regards, the invention relates to novel polynucleotides and polypeptides of the Glu-tRNA^{Gln} Amidotransferase family, hereinafter referred to as "Glu-tRNA^{Gln} AdT" or "AdT". The present invention further provides methods and compositions for use in identifying and using protein translation inhibitors as antibacterial, antifungal or herbicidal agents.

BACKGROUND OF THE INVENTION

Prior to their incorporation into protein, amino acids are chemically linked to small RNA molecules called transfer RNA (tRNA). For each of the 20 different amino acids, a specific enzyme catalyzes its linkage to the 3' end of its specific tRNA molecule. While the general mechanism of protein biosynthesis (the translation

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process) is conserved throughout the living kingdom there exist two different pathways for the formation of Gln-tRNA^{Gln}. While the two pathways for Gln-tRNA^{Gln} formation are evolutionarily conserved, the reason for existence of the different pathways is as yet not known. In gram-negative eubacteria and in the cytoplasm of eukaryotic cells the enzyme glutamyl-tRNA synthetase (GlnRS) acylates glutamine directly to the cognate tRNA to provide Gln-tRNA^{Gln}. Interestingly, GlnRS is not detectable in several biological systems. In certain organisms and organelles including the archae, gram-positive eubacteria, mitochondria and chloroplasts a different pathway of Gln-tRNA^{Gln} formation, a transamidation pathway is operative (Curnow *et al.* (1996) *Nature* 382: 589-590; Curnow *et al.* (1997) *Proc. Natl. Acad. Sci. USA* 94(22):11819-11826; Schön *et al.* (1988) *Biochimie* 70(3):391-394; Wilcox & Nirenberg (1968) *Proc. Natl. Acad. Sci. USA* 61(1):229-236; Schön *et al.* (1988) *Nature* 331:187-190. This pathway (depicted in Figure 1) is initiated by misacylation of tRNA^{Gln} by glutamyl-tRNA synthetase (GluRS) forming Glu-tRNA^{Gln}. The incorrectly charged tRNA is then converted to Gln-tRNA^{Gln} by Glu-tRNA^{Gln} amidotransferase (AdT). AdT catalyzes the amidation of glutamate to glutamine only when the glutamate is covalently attached to tRNA^{Gln}. It has been shown that the partially purified Glu-tRNA^{Gln} amidotransferase activity from *Bacillus megaterium* in the presence of ATP, Mg⁺⁺, and an amide-nitrogen donor (glutamine) will carry out the amidation of Glu-tRNA^{Gln} to Gln-tRNA^{Gln} (Wilcox & Nirenberg, 1968).

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Subsequent work demonstrated, in vitro, that the amidation proceeds through the activated intermediate (phospho-GlutRNA^{Gln}) (Wilcox (1969) *Cold Spring Harb. Symp. Quant. Biol.* 34:521-528; Wilcox (1969) *Eur J. Biochem* 11(3):405-412). Since the initial aminoacylation product, GlutRNA^{Gln}, would be toxic to the cell due to the
5 fact that it would result in faulty protein translation, it must be converted to the correctly charged tRNA.

It appears that this pathway is the primary source of Gln-tRNA^{Gln} within these cells and may act as a regulatory mechanism for glutamine metabolism. Evolutionarily, it has been suggested that glutamine was the last amino acid formed.
10 Therefore it may be postulated that cells which employ the transamidation pathway utilized the gene encoding GluRS to generate the AdT. Likewise, in the cells in which the direct glutaminylation pathway operates, the enzyme GlnRS may have evolved from a GluRS gene duplication (Rogers & Söll (1995) *J. Mol. Evol.* 40 (5) p476-81). This is reasonable since both enzymes are required to specifically
15 recognize and bind tRNA^{Gln} and free glutamine. However, database searches and, in particular, a detailed analysis of the Mycoplasma genome (Fraser et al. (1995) *Science* 270(5235):397-403), the only gram-positive organism sequenced and published to date, have shown no significant homologies to GluRS and GlnRS in the currently
20 available sequence information. Thus, the amidotransferase may not have significant homology to the aminoacyl-tRNA synthetases. Despite the unquestioned

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evolutionary and biochemical significance in understanding this system, there have been very few investigations of this enzyme to date (Wilcox & Nirenberg, 1968; Wilcox, 1969; Strauch et al. (1988) *J. Bacteriol.* 170:916-920; and Jahn (1990) *J. Biol. Chem.* 265(14):8059-64).

5 SUMMARY OF THE INVENTION

The present invention is based, in part, on the isolation and characterization of a heterotrimeric protein designated AdT that is involved in generating GlutRNA^{Gln} from GlutRNA^{Gln}. This invention further provides polypeptides that have been identified as novel AdT polypeptides by homology between the amino acid sequence of GlutRNA^{Gln} AdT and a known amino acid sequence.

This invention further provides polynucleotides that encode AdT polypeptides. In particular, this invention provides the polynucleotide sequence encoding GlutRNA^{Gln} AdT comprising the sequence set out in Figure 3 (SEQ ID NO:1), or a variant thereof, such as naturally occurring allelic variants of AdT and polypeptides encoded thereby. Thus, this invention provides polynucleotides that hybridize to AdT polynucleotide sequences, particularly under stringent conditions.

This invention provides GlutRNA^{Gln} AdT protein from *B. subtilis* comprising the amino acid sequences encoded by the nucleotide sequence of Figure 3 (SEQ ID NOS:1, 3, 5 and 7), as well as biologically, diagnostically, prophylactically, clinically

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or therapeutically useful variants thereof, and compositions comprising the same. Particularly preferred variants include AdT polypeptides encoded by naturally occurring alleles of the AdT gene. Methods for producing the aforementioned AdT polypeptides are also provided by this invention.

5 The invention also provides isolated nucleic acid molecules encoding AdT, particularly *B. subtilis* AdT, including mRNAs, cDNAs, and genomic DNAs, including biologically, diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and compositions comprising the same.

 In accordance with yet another aspect of the invention, there are provided
10 inhibitors to such AdT polypeptides, useful as antibacterial agents, antifungal agents and herbicides. Thus, the present invention provides compositions and methods for use in identifying agonists and antagonists of the AdT protein.

 This invention provides compositions and methods for (i) assessing AdT expression, (ii) treating disease, for example, diseases associated with excessive or
15 deficient amounts of available AdT, (iii) assaying genetic variation, and (iv) and administering an AdT polypeptide or polynucleotide to a cell or to a multicellular organism to raise an immunological response. In certain preferred embodiments of this aspect of the invention there are provided antibodies against AdT polypeptides.

 This invention also provides compositions and methods for protecting plants,
20 especially crop plants. For example, this invention provides antagonists of AdT

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which are useful as herbicides, as well as the herbicidal compositions which include such inhibitors of AdT. This invention also provides non-inhibited mutants of AdT and functional derivatives thereof which are resistant to inhibition from certain herbicides, especially herbicides containing inhibitors of AdT. The polynucleotides coding for the non-inhibited AdT can be placed in plants by various transformation methods so as to render the plants tolerant or resistant to certain herbicides containing inhibitors of AdT. Therefore, methods of treating weeds utilizing the application of AdT inhibitors to transgenic plants containing the non-inhibited mutants of AdT are also encompassed by this invention.

10 Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the transamidation pathway for the formation of GlntRNA^{Gln}.

15 Figure 2 shows the gene arrangement of the AdT gene.

Figure 3 shows the nucleic acid sequence of the AdT protein from *B. subtilis*.

DESCRIPTION OF THE INVENTION

I. General Description

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The present invention is based, in part, on the identification and characterization of a heterotrimeric protein that is responsible for generating Gln^tRNA within a cell, herein after the AdT protein. The present invention specifically provides the amino acid sequences of each of the three subunits of an AdT protein isolated from *B. subtilis*, as well as nucleotide sequences that encode the AdT protein. The AdT protein and nucleic acid molecules can serve as targets in methods for identifying agents for use in inhibiting protein synthesis, particularly antimicrobial, antifungal and herbicide agents.

II. Specific Embodiments

10 A. AdT Protein

Prior to the present invention the art had taught that there was an enzyme involved in converting GlutRNA^{Gln} to Gln^tRNA^{Gln}. However the isolation and characterization of the protein responsible for generating Gln^tRNA remained unknown. The present invention provides, in part, the amino acid sequences of the three subunits of the *B. subtilis* AdT protein. Quite unexpectedly, this AdT protein was found to be a heterotrimeric protein.

In one embodiment, the present invention provides the ability to produce a previously unknown protein using the cloned nucleic acid molecules herein described or by synthesizing a protein having the amino acid sequence herein disclosed.

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As used herein, the AdT protein refers to a protein that has the amino acid sequence *B. subtilis* AdT encoded by the polynucleotide of Fig. 1, allelic variants thereof and conservative substitutions thereof that have AdT activity. The AdT protein is comprised of 3 subunits: the A (SEQ ID NO:4), B (SEQ ID NO:6) and C (SEQ ID NO:8) subunits, referred to herein collectively as aAdT, bAdT and cAdT subunits, respectively. For the sake of convenience, the collective subunits will be referred to as the AdT protein or the AdT protein of the present invention. A skilled artisan can readily recognize within the context whether a single subunit or the collective protein is being referred to.

10 The polypeptides of the invention include the polypeptides encoded by SEQ ID NO:1 (Figure 3) as well as polypeptides and fragments, particularly those which have the biological activity of AdT and also those which have at least 70% sequence identity to the polypeptides encoded by SEQ ID NO:1 or the relevant portion, preferably at least 80% identity to the polypeptides encoded by SEQ ID NO:1, and
15 more preferably at least 90% similarity (more preferably at least 90% identity) to the polypeptides encoded by SEQ ID NO:1 and still more preferably at least 95% similarity (still more preferably at least 95% identity) to the polypeptides encoded by SEQ ID NO:1 and also include portions of such polypeptides.

The AdT proteins of the present invention include the specifically identified
20 and characterized variant herein described as well as allelic variants, conservative

substitution variants and homologues that can be isolated/generated and characterized without undue experimentation following the methods outlined below. For the sake of convenience, all AdT proteins will be collectively referred to as the AdT proteins or the AdT proteins of the present invention.

5 The term "AdT proteins" includes all naturally occurring allelic variants of the *B. subtilis* AdT protein that possess normal AdT activity. In general, allelic variants of the AdT protein will have a slightly different amino acid sequence than that specifically encoded by SEQ ID NO:1 but will be able to convert GlutRNA to GlnRNA. Allelic variants, though possessing a slightly different amino acid
10 sequence than those recited above, will possess the ability to generate GlnRNA. Typically, allelic variants of the AdT protein will contain conservative amino acid substitutions from the AdT sequences herein described or will contain a substitution of an amino acid from a corresponding position in an AdT homologue (an AdT protein isolated from an organism other than *B. subtilis*).

15 The AdT proteins of the present invention are preferably in isolated form. As used herein, a protein is said to be isolated when physical, mechanical or chemical methods are employed to remove the AdT protein from cellular constituents that are normally associated with the protein. A skilled artisan can readily employ standard purification methods to obtain an isolated AdT protein. One purification scheme is

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outlined in Example 1. The nature and degree of isolation will depend on the intended use.

The cloning of an AdT encoding nucleic acid molecule makes it possible to generate defined fragments of the AdT proteins of the present invention. As discussed
5 below, fragments of the AdT proteins of the present invention are particularly useful in generating subunit specific antibodies, in identifying agents that bind to a AdT protein and in isolating homologues of the *B. subtilis* AdT protein.

Fragments of the AdT proteins can be generated using standard peptide synthesis technology and the amino acid sequences disclosed herein. Alternatively,
10 recombinant methods can be used to generate nucleic acid molecules that encode a fragment of the AdT protein.

Fragments of the AdT protein subunits that contain particularly interesting structures can be identified using art-known methods such as immunogenicity, Chou-Fasman, Garnier-Robson, Kyte-Doolittle, Eisenberg, Karplus-Schultz or
15 Jameson-Wolf analysis. Fragments containing such residues are particularly useful in generating subunit specific anti-AdT antibodies.

As described below, members of the AdT family of proteins can be used for, but are not limited to: 1) a target to identify agents that block or stimulate AdT activity, 2) a target or bait to identify and isolate binding partners that bind an AdT

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protein, 3) identifying agents that block or stimulate the activity of an AdT protein and
4) an assay target to identify AdT mediated activity or disease.

B. Anti-AdT Antibodies

The present invention further provides antibodies that selectively bind one or
5 more of the AdT proteins of the present invention, or to a specific subunit of an AdT
protein of the present invention. The most preferred antibodies will bind to either an
entire heterotrimeric AdT protein but not to an isolated subunit or will bind to an
isolated subunit but not to the assembled trimeric protein. Anti- AdT antibodies that
are particularly contemplated include monoclonal and polyclonal antibodies as well as
10 fragments containing the antigen binding domain and/or one or more complement
determining regions of these antibodies.

Antibodies are generally prepared by immunizing a suitable mammalian host
using an AdT protein, or fragment, in isolated or immunoconjugated form (Harlow,
Antibodies, Cold Spring Harbor Press, NY (1989)). Regions of the AdT protein that
15 show immunogenic structure can readily be identified using art-known methods.
Other important regions and domains can readily be identified using protein analytical
and comparative methods known in the art.

Fragments containing these residues are particularly suited in generating
specific classes of anti-AdT antibodies.

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Methods for preparing a protein for use as an immunogen and for preparing immunogenic conjugates of a protein with a carrier such as BSA, KLH, or other carrier proteins are well known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents may be used; in other instances linking
5 reagents such as those supplied by Pierce Chemical Co., Rockford, IL, may be effective.

Administration of an AdT immunogen is conducted generally by injection over a suitable time period and with use of a suitable adjuvant, as is generally understood in the art. During the immunization schedule, titers of antibodies can be
10 taken to determine adequacy of antibody formation.

While the polyclonal antisera produced in this way may be satisfactory for some applications, for pharmaceutical compositions, monoclonal antibody preparations are preferred. Immortalized cell lines which secrete a desired monoclonal antibody may be prepared using the standard method of Kohler and
15 Milstein or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known. The immortalized cell lines secreting the desired antibodies are screened by immunoassay in which the antigen is the AdT protein or peptide fragment. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells can be cultured either in vitro or by production
20 in ascites fluid.

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The desired monoclonal antibodies are then recovered from the culture supernatant or from the ascites supernatant. Fragments of the monoclonals or the polyclonal antisera which contain the immunologically significant portion can be used as antagonists, as well as the intact antibodies. Use of immunologically reactive
5 fragments, such as the Fab, Fab', of F(ab')₂ fragments is often preferable, especially in a therapeutic context, as these fragments are generally less immunogenic than the whole immunoglobulin.

The antibodies or fragments may also be produced, using current technology, by recombinant means. Regions that bind specifically to the desired regions of the
10 transporter can also be produced in the context of chimeric or CDR grafted antibodies of multiple species origin.

As described below, anti-AdT antibodies are useful as modulators of AdT activity, are useful in immunoassays for detecting AdT expression/activity and for purifying homologues of the *B. subtilis* AdT protein.

15 C. AdT Encoding Nucleic Acid Molecules

As described above, the present invention is based, in part, on isolating nucleic acid molecules from *B. subtilis* that encode the three subunits of the AdT protein. Accordingly, the present invention further provides nucleic acid molecules that encode the AdT protein, as herein defined, preferably in isolated form. For

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convenience, all AdT encoding nucleic acid molecules will be referred to as AdT encoding nucleic acid molecules, the AdT genes, or AdT. The nucleotide sequence of the *B. subtilis* nucleic acid molecule that encodes each of the subunits of AdT is provided in SEQ ID NO:1. The start and stop codons for each of subunits A (SEQ ID NO:4), B (SEQ ID NO:6) and C (SEQ ID NO:8) are designated in the nucleotide sequence for AdT provided in Figure 3.

Further preferred embodiments of the invention are polynucleotides that are at least 70% sequence identical over their entire length to a polynucleotide encoding AdT polypeptides having an amino acid sequence encoded by SEQ ID NO:1, and polynucleotides which are complementary to such polynucleotides. Alternatively, most highly preferred are polynucleotides that comprise a region that is at least 80% identical over their entire length to a polynucleotide encoding AdT polypeptide and polynucleotides complementary thereto. In this regard, polynucleotides at least 90% identical over their entire length to the same are particularly preferred, and among these particularly preferred polynucleotides, those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

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The invention further relates to variants of the herein above described polynucleotides which encode for variants of the polypeptides having the deduced amino acid sequences of SEQ ID NO:1.

5 Variants that are fragments of the polypeptides of the invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, these variants may be employed as intermediates for producing the full-length polypeptides. Variants that are fragments of the polynucleotides of the invention may be used to synthesize full-length polynucleotides of the invention. Variants that are fragments of the polynucleotides of the invention may be used to
10 synthesize full-length polynucleotides of the invention. Such methods are widely available, such as those disclosed in WO 97/26340 and WO 97/38716.

A fragment is a variant polypeptide having an amino acid sequence that entirely is the same as part but not all of the amino acid sequence of the aforementioned polypeptides. AdT polypeptides fragments may be "free-standing,"
15 or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region, a single larger polypeptide.

Further particularly preferred embodiments are polynucleotides encoding AdT variants, which have the amino acid sequence of the AdT polypeptides encoded by SEQ ID NO:1 in which several, a few, 10 to 15, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no
20 amino acid residues are substituted, deleted or added, in any combination. Especially

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preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of AdT.

As used herein, a "nucleic acid molecule" is defined as an RNA or DNA molecule that encodes a peptide as defined above, or is complementary to a nucleic acid sequence encoding such peptides. Particularly preferred nucleic acid molecules will have a nucleotide sequence identical to or complementary to the *B. subtilis* DNA sequences herein disclosed. Specifically contemplated are genomic DNA, polycistronic mRNA and antisense molecules, as well as nucleic acids based on an alternative backbone or including alternative bases, whether derived from natural sources or synthesized. Such nucleic acid molecules, however, are defined further as being novel and unobvious over any prior art nucleic acid molecules encoding non-AdT proteins isolated from organisms other than *B. subtilis*.

As used herein, a nucleic acid molecule is said to be "isolated" when the nucleic acid molecule is substantially separated from contaminant nucleic acid molecules that encode polypeptides other than AdT. A skilled artisan can readily employ nucleic acid isolation procedures to obtain an isolated AdT encoding nucleic acid molecule.

The present invention further provides fragments of the AdT encoding nucleic acid molecules of the present invention. As used herein, a fragment of an AdT encoding nucleic acid molecule refers to a small portion of the entire protein encoding

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sequence. The size of the fragment will be determined by its intended use. For example, if the fragment is chosen so as to encode an active portion of the AdT protein, such an active domain or effector binding site, then the fragment will need to be large enough to encode the functional region(s) of the AdT protein. If the fragment is to be used as a nucleic acid probe or PCR primer, then the fragment length is chosen so as to obtain a relatively small number of false positives during probing/priming. Fragments of the *B. subtilis* AdT nucleic acid molecule that are particularly useful as selective hybridization probes or PCR can be readily determined using art-known methods.

10 Fragments of the AdT encoding nucleic acid molecules of the present invention (i.e., synthetic oligonucleotides) that are used as probes or specific primers for the polymerase chain reaction (PCR), or to synthesize gene sequences encoding AdT proteins, can easily be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci, et al., *J Am Chem Soc* (1981) 103:3185-3191 or
15 using automated synthesis methods. In addition, larger DNA segments can readily be prepared by well known methods, such as synthesis of a group of oligonucleotides that define various modular segments of the AdT gene, followed by ligation of oligonucleotides to build the complete modified AdT gene.

 The AdT encoding nucleic acid molecules of the present invention may further
20 be modified so as to contain a detectable label for diagnostic and probe purposes. As

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described above, such probes can be used to identify nucleic acid molecules encoding other allelic variants or homologues of the AdT proteins and as described below, such probes can be used to diagnose the presence of a AdT protein as a means for diagnosing a pathological condition caused by AdT mediated translation. A variety of such labels are known in the art and can readily be employed with the AdT encoding molecules herein described. Suitable labels include, but are not limited to, biotin, radiolabeled nucleotides, biotin, and the like. A skilled artisan can employ any of the art-known labels to obtain a labeled AdT encoding nucleic acid molecule.

D. Isolation of Other AdT Encoding Nucleic Acid Molecules

The identification of the AdT protein from *B. subtilis* and the corresponding nucleic acid molecules, has made possible the identification of and isolation of AdT proteins from organisms other than *B. subtilis*, hereinafter referred to collectively as AdT homologues. The preferred source of the AdT homologues are pathogenic microorganisms such as bacteria and fungi, as well as plants in which it is desirable to control growth. The most preferred sources are gram positive bacteria, pathogenic fungi and plant organelles such as chloroplasts.

Essentially, a skilled artisan can readily use the amino acid sequence of the *B. subtilis* AdT protein to generate antibody probes to screen expression libraries prepared from cells. Typically, polyclonal antiserum from mammals such as rabbits

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immunized with the purified protein (as described below) or monoclonal antibodies can be used to probe an expression library, prepared from a target organism, to obtain the appropriate coding sequence for AdT protein homologue. The cloned cDNA sequence can be expressed as a fusion protein, expressed directly using its own
5 control sequences, or expressed by constructing an expression cassette using control sequences appropriate to the particular host used for expression of the enzyme.

Alternatively, a portion of the AdT encoding sequence herein described can be synthesized and used as a probe to retrieve DNA encoding a member of the AdT family of proteins from organisms other than *B. subtilis*. Oligomers containing
10 approximately 18-20 nucleotides (encoding about a 6-7 amino acid stretch) are prepared and used to screen genomic DNA or cDNA libraries to obtain hybridization under stringent conditions or conditions of sufficient stringency to eliminate an undue level of false positives. This method can be used to identify and isolate altered and variants of the AdT encoding sequences.

15 Additionally, pairs of oligonucleotide primers can be prepared for use in a polymerase chain reaction (PCR) to selectively amplify/clone an AdT-encoding nucleic acid molecule, or fragment thereof. A PCR denature/anneal/extend cycle for using such PCR primers is well known in the art and can readily be adapted for use in isolating other AdT encoding nucleic acid molecules. Regions of the *B. subtilis* AdT
20 gene that are particularly well suited for use as a probe or as primers can be readily

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identified. In general, the preferred primers will flank one or more of the subunit encoding regions of the *B. subtilis* AdT gene.

Homologues of the herein disclosed AdT proteins will share homology. In general, nucleic acid molecules that encode AdT homologues will hybridize to the
5 *B. subtilis* sequences under high stringency. Such sequences will typically contain at least 70% homology, preferably at least 80%, most preferably at least 90% homology to the *B. subtilis* sequences.

E. Recombinant DNA Molecules Containing an AdT Encoding Nucleic Acid Molecule

10 The present invention further provides recombinant DNA molecules that contain one or more of the AdT encoding sequences herein described, or a fragment of the herein-described nucleic acid molecules. As used herein, a recombinant DNA molecule is a DNA molecule that has been subjected to molecular manipulation in vitro. Methods for generating recombinant DNA molecules are well known in the art,
15 for example, see Sambrook et al., *Molecular Cloning* (1989). In the preferred recombinant DNA molecules, an AdT encoding DNA sequence that encodes an AdT protein, or AdT subunit is operably linked to one or more expression control sequences and/or vector sequences. The recombinant DNA molecule can encode

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either a single subunit of the AdT protein, or can encode an operon that contains all three of the AdT subunits.

The choice of vector and/or expression control sequences to which one of the AdT encoding sequences of the present invention is operably linked depends directly, as is well known in the art, on the functional properties desired, e.g., protein expression, and the host cell to be transformed. A vector contemplated by the present invention is at least capable of directing the replication or insertion into the host chromosome, and preferably also expression, of an AdT encoding sequence included in the recombinant DNA molecule.

Expression control elements that are used for regulating the expression of an operably linked protein encoding sequence are known in the art and include, but are not limited to, inducible promoters, constitutive promoters, secretion signals, enhancers, transcription terminators and other regulatory elements. Preferably, an inducible promoter that is readily controlled, such as being responsive to a nutrient in the host cell's medium, is used.

In one embodiment, the vector containing an AdT encoding nucleic acid molecule will include a prokaryotic replicon, i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule intrachromosomally in a prokaryotic host cell, such as a bacterial host cell, transformed therewith. Such replicons are well known in the art. In addition, vectors

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that include a prokaryotic replicon may also include a gene whose expression confers a detectable marker such as a drug resistance. Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline.

5 Vectors that include a prokaryotic replicon can further include a prokaryotic or viral promoter capable of directing the expression (transcription and translation) of the AdT encoding sequence in a bacterial host cell, such as *E. coli*. A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites
10 for insertion of a DNA segment of the present invention. Typical of such vector plasmids are pUC8, pUC9, pBR322 and pBR329 available from Biorad Laboratories (Richmond, CA), pPL and pKK223 available from Pharmacia, Piscataway, NJ.

Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can also be used to variant recombinant DNA
15 molecules that contain an AdT encoding sequence. Eukaryotic cell expression vectors are well known in the art and are available from several commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of the desired DNA segment. Typical of such vectors are PSVL and pKSV-10 (Pharmacia), pBPV-1/pML2d (International Biotechnologies, Inc.), pTDT1

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(ATCC, #31255), the vector pCDM8 described herein, and the like eukaryotic expression vectors.

Eukaryotic cell expression vectors used to construct the recombinant DNA molecules of the present invention may further include a selectable marker that is effective in an eukaryotic cell, preferably a drug resistance selection marker. A preferred drug resistance marker is the gene whose expression results in neomycin resistance, i.e., the neomycin phosphotransferase (neo) gene. Southern et al., *J Mol Anal Genet* (1982) 1:327-341. Alternatively, the selectable marker can be present on a separate plasmid, and the two vectors are introduced by cotransfection of the host cell, and selected by culturing in the presence of the appropriate drug for the selectable marker.

F. Host Cells Containing an Exogenously Supplied AdT Encoding Nucleic Acid Molecule

The present invention further provides host cells transformed with a nucleic acid molecule that encodes an AdT protein of the present invention, either the entire heterotrimeric protein or one or more subunits. The host cell can be either prokaryotic or eukaryotic. Eukaryotic cells useful for expression of an AdT protein are not limited, so long as the cell line is compatible with cell culture methods and compatible with the propagation of the expression vector and expression of an AdT

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gene. Preferred eukaryotic host cells include, but are not limited to, yeast, insect and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human fibroblastic cell line, the most preferred being cells that do not naturally express an AdT protein.

- 5 Any prokaryotic host can be used to express an AdT-encoding recombinant DNA molecule. The preferred prokaryotic host is *E. coli*.

Transformation of appropriate cell hosts with an recombinant DNA molecule of the present invention is accomplished by well known methods that typically depend on the type of vector used and host system employed. With regard to transformation
10 of prokaryotic host cells, electroporation and salt treatment methods are typically employed, see, for example, Cohen et al., *Proc Acad Sci USA* (1972) 69:2110; and Maniatis et al., *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982). With regard to transformation of
vertebrate cells with vectors containing recombinant DNAs, electroporation, cationic
15 lipid or salt treatment methods are typically employed, see, for example, Graham et al., *Virol* (1973) 52:456; Wigler et al., *Proc Natl Acad Sci USA* (1979) 76:1373-76.

Successfully transformed cells, i.e., cells that contain an recombinant DNA molecule of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an recombinant DNA of the present
20 invention can be cloned to produce single colonies. Cells from those colonies can be

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harvested, lysed and their DNA content examined for the presence of the recombinant DNA using a method such as that described by Southern, *J Mol Biol* (1975) 98:503, or Berent et al., *Biotech* (1985) 3:208 or the proteins produced from the cell assayed via an immunological method.

5 G. Production of an AdT Protein Using an recombinant DNA Molecule

The present invention further provides methods for producing an AdT protein that uses one of the AdT encoding nucleic acid molecules herein described. In general terms, the production of a recombinant AdT protein typically involves the following steps.

- 10 First, a nucleic acid molecule is obtained that encodes an AdT protein, such as the nucleic acid molecule depicted in Figure 3 (SEQ ID NO:1) or an AdT subunit. The AdT encoding nucleic acid molecule is then preferably placed in an operable linkage with suitable control sequences, as described above, to generate an expression unit containing the AdT encoding sequence. The expression unit is used to transform
- 15 a suitable host and the transformed host is cultured under conditions that allow the production of the AdT protein. Optionally the AdT protein is isolated from the medium or from the cells; recovery and purification of the protein may not be necessary in some instances where some impurities may be tolerated.

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Each of the foregoing steps can be done in a variety of ways. For example, the desired coding sequences may be obtained from genomic fragments and used directly in an appropriate host. The construction of expression vectors that are operable in a variety of hosts is accomplished using an appropriate combination of replicons and control sequences. The control sequences, expression vectors, and transformation methods are dependent on the type of host cell used to express the gene and were discussed in detail earlier. Suitable restriction sites can, if not normally available, be added to the ends of the coding sequence so as to provide an excisable gene to insert into these vectors. A skilled artisan can readily adapt any host/expression system known in the art for use with AdT encoding sequences to produce an AdT protein.

H. Identification of Agents that Bind to an AdT Protein

Another embodiment of the present invention provides methods for identifying agents that are agonists or antagonists of the AdT proteins herein described. Specifically, agonists and antagonists of an AdT protein can be identified by the ability of the agent to bind to an AdT protein and/or the ability to inhibit AdT activity. Activity assays for AdT activity and binding assays using an AdT protein are suitable for use in high through-put screening methods.

In detail, in one embodiment, an AdT protein is mixed with an agent. After mixing under conditions that allow association of AdT protein with the agent, the

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mixture is analyzed to determine if the agent bound the AdT protein. Agonists and antagonists are identified as being able to bind to an AdT protein. Alternatively or consecutively, as described below, AdT activity can be directly assessed as a means for identifying agonists and antagonists of AdT activity.

- 5 The AdT protein used in the above assay can be: an isolated and fully characterized protein, a single subunit of an AdT protein, a partially purified protein, a cell that has been altered to express an AdT protein or a fraction of a cell that has been altered to express an AdT protein. Further, the AdT protein can be the entire AdT protein, a specific fragment of the AdT protein or a single subunit of the AdT protein.
- 10 It will be apparent to one of ordinary skill in the art that so long as the AdT protein can be assayed for agent binding, e.g., by a shift in molecular weight or activity, as described in the Examples, the present assay can be used. The AdT protein is particularly well suited for high through-put screening methods.

- The source of the AdT protein will be based on the intended use of the
- 15 modulating agent. For example, microbial AdT protein is used to identify AdT inhibitors that have bactericidal activity whereas chloroplast derived AdT protein is used to identify AdT inhibitors that have herbicide activity.

- The method used to identify whether an agent binds to an AdT protein will be based primarily on the nature of the AdT protein used. For example, a gel retardation
- 20 assay can be used to determine whether an agent binds to a soluble fragment of an

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AdT protein. Alternatively, immunodetection and biochip technologies can be adopted for use with an AdT protein. A skilled artisan can readily employ numerous art-known techniques for determining whether a particular agent binds to an AdT protein.

5 Agents can be further tested for the ability to modulate the activity of an AdT protein using a cell-free assay system or a cellular assay system. Example 1 provides one such methods that can be used to assay for AdT activity.

 As used herein, an agent is said to antagonize AdT activity when the agent reduces AdT activity. The preferred antagonist will selectively antagonize AdT, not
10 affecting any other cellular proteins, particularly other proteins involved in translation. Further, the preferred antagonist will reduce AdT activity by more than 50%, more preferably by more than 90%, most preferably eliminating all AdT activity.

 As used herein, an agent is said to agonize AdT activity when the agent increases AdT activity. The preferred agonist will increase AdT activity by more than
15 50%, more preferably by more than 90%, most preferably more than doubling the level of AdT activity.

 The preferred antagonists and agonists will be selective for a specific species, genus, family, order or kingdom of organisms. Agents can be screened using one AdT protein, or a combination of AdT proteins, to aid in identifying agents for target
20 specificity. For example, several different microbial AdT proteins can be used to

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identify general antimicrobial agents whereas chloroplast derived AdT proteins can be used to identify herbicide agents.

Agents that are assayed in the above method can be randomly selected or rationally selected or designed. As used herein, an agent is said to be randomly
5 selected when the agent is chosen randomly without considering the specific sequences of the AdT protein. An example of randomly selected agents is the use a chemical library or a peptide combinatorial library, or a growth broth of an organism or plant extract.

As used herein, an agent is said to be rationally selected or designed when the
10 agent is chosen on a nonrandom basis that takes into account the sequence of the target site and/or its conformation in connection with the agent's action. Agents can be rationally selected or rationally designed by utilizing the peptide sequences that make up the AdT protein. For example, a rationally selected peptide agent can be a peptide whose amino acid sequence is identical to a fragment of an AdT protein.

15 The agents of the present invention can be, as examples, peptides, small molecules, and vitamin derivatives, as well as carbohydrates. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents of the present invention. One class of agents of the present invention are peptide agents whose amino acid sequences are chosen based on the amino acid sequence of the AdT

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protein. Small peptide agents can serve as competitive inhibitors of AdT protein assembly.

The peptide agents of the invention can be prepared using standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the DNA encoding these peptides may be synthesized using commercially available
5 oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production using solid phase peptide synthesis is necessitated if non-gene-encoded amino acids are to be included.

Another class of agents of the present invention are antibodies immunoreactive
10 with critical positions of the AdT protein. As described above, antibodies are obtained by immunization of suitable mammalian subjects with peptides, containing as antigenic regions, those portions of the AdT protein intended to be targeted by the antibodies. Critical regions include the domains identified in Figure 2. Such agents can be used in competitive binding studies to identify second generation inhibitory
15 agents.

K. Uses of Agents that Bind to an AdT Protein

As provided in the Background section, the AdT proteins are involved in protein translation, particularly protein translation in gram positive microorganisms, fungi and cellular organelles, particularly chloroplasts. Agents that bind an AdT

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protein and act as an agonist or antagonist can be used to modulate translation in these organism and serves as a basis for an antibacterial, antifungal or herbicide agents. In detail, protein translation that requires AdT can be modulated by administering to an organism an agent that binds to an AdT protein and acts as an agonist or antagonist of

5 AdT activity.

As used herein, an organism can be any organism, so long as it is desirable to modulate protein translation in the organism, for example to control the growth of an infectious agent in a mammalian subject or to act as an herbicide agent. The invention is particularly useful in the treatment of human subjects for controlling microbial

10 growth.

As used herein, protein translation that requires AdT refers to protein translation that would not occur without the presence of an active AdT protein. As used herein, an agent is said to modulate AdT mediated protein translation when the agent reduces the degree of protein translation.

15 The use of the AdT modulating agents will be based primarily on the target AdT protein used to identify the agent as well as the activity/selectivity of the agent. For example, an AdT inhibitory agent, that is used as an antimicrobial agent, is preferably isolated using one or more microbial AdT proteins. Herbicide agent will be preferably identified using chloroplast AdT protein as a target.

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L. Administration of Agonists and Antagonists of an AdT Protein

The administration of agonists and antagonists of the AdT protein will be dependent on their intended purpose. For example, to control microbial growth in a mammalian subject, an AdT inhibitory agent can be administered via parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, or buccal routes. Alternatively, or concurrently, administration may be by the oral route. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. For example, to treat microbial infection, an agent that modulates AdT activity is administered systemically or locally to the individual being treated. As described below, there are many methods that can readily be adapted to administer such agents.

The present invention further provides compositions containing an antagonist or agonist of an AdT protein that is identified by the methods herein described. The determination of optimal ranges of effective amounts of each component is within the skill of the art and is based on the intended use.

In addition to the AdT modulating agent, the compositions of the present invention may contain other ingredients, such as suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically for delivery to the

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site of action. Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble variant, for example, water-soluble salts. In addition, suspensions of the active compounds and as appropriate, oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles
5 include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension and include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dintran. Optionally, the suspension may also contain stabilizers. Liposomes can also be used to encapsulate the agent for
10 delivery into the cell.

The pharmaceutical formulation for systemic administration according to the invention may be formulated for enteral, parenteral or topical administration. Indeed, all three types of formulations may be used simultaneously to achieve systemic administration of the active ingredient.

15 Suitable formulations for oral administration include hard or soft gelatin capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and controlled release variants thereof.

M. Combination Therapy

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The agents of the present invention that modulate AdT activity can be provided alone, or in combination with another agents that modulate protein synthesis microbial, fungal or plant growth. For example, an agent of the present invention that reduces microbial AdT activity can be administered in combination with other
5 antimicrobial agents. As used herein, two agents are said to be administered in combination when the two agents are administered simultaneously or are administered independently in a fashion such that the agents will act at the same time.

N. Methods for Identifying the Presence of an AdT protein or gene

The present invention further provides methods for identifying cells or
10 organisms expressing an AdT protein or an AdT gene. Such methods can be used to diagnose the presence of an organism that expresses an AdT protein. The methods of the present invention are particularly useful in the determining the presence of pathogenic microorganisms. Specifically, the presence of an AdT protein can be identified by determining whether an AdT protein, or nucleic acid encoding an AdT
15 protein, is expressed. The expression of an AdT protein can be used as a means for diagnosing the presence of an organism that relies on AdT mediated translation.

A variety of immunological and molecular genetic techniques can be used to determine if an AdT protein is expressed/produced in a particular cell or sample. In general, an extract containing nucleic acid molecules or an extract containing proteins

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is prepared. The extract is then assayed to determine whether an AdT protein, or an AdT encoding nucleic acid molecule, is produced in the cell.

For example, to perform a diagnostic test based on nucleic acid molecules, a suitable nucleic acid sample is obtained and prepared using conventional techniques.

5 DNA can be prepared, for example, simply by boiling a sample in SDS. The extracted nucleic acid can then be subjected to amplification, for example by using the polymerase chain reaction (PCR) according to standard procedures, to selectively amplify an AdT encoding nucleic acid molecule or fragment thereof. The size or presence of a specific amplified fragment (typically following restriction
10 endonuclease digestion) is then determined using gel electrophoresis or the nucleotide sequence of the fragment is determined (for example, see Weber and May *Am J Hum Genet* (1989) 44:388-339; Davies, J. et al. *Nature* (1994) 371:130-136)). The resulting size of the fragment or sequence is then compared to the known AdT proteins encoding sequences, for example via hybridization probe. Using this
15 method, the presence of an AdT protein can be identified.

To perform a diagnostic test based on proteins, a suitable protein sample is obtained and prepared using conventional techniques. Protein samples can be prepared, for example, simply by mixing a sample with SDS followed by salt precipitation of a protein fraction. The extracted protein can then be analyzed to
20 determine the presence of an AdT protein using known methods. For example, the

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presence of specific sized or charged variants of a protein can be identified using mobility in an electric field. Alternatively, antibodies can be used for detection purposes. A skilled artisan can readily adapt known protein analytical methods to determine if a sample contains an AdT protein.

5 Alternatively, AdT expression can also be used in methods to identify agents that decrease the level of expression of the AdT gene. For example, cells or tissues expressing an AdT protein can be contacted with a test agent to determine the effects of the agent on AdT expression. Agents that activate AdT expression can be used as an agonist of AdT activity whereas agents that decrease AdT expression can be used
10 as an antagonist of AdT activity.

O. Preparation and Use of Herbicides

As discussed herein, the transamidation pathway is operative in chloroplasts. The ability to identify AdT inhibitors which specifically inhibit plastid isoforms of AdT can be useful in designing herbicides that are not toxic or harmful to humans and
15 animals. Thus, the ability to develop herbicides that inhibit only chloroplast isoforms of enzymes such as Adt but do not inhibit cytosolic (i.e., the fluid portion of the cytoplasm exclusive of organelles) AdT or human AdT, would provide a new form of highly effective herbicide that is also less toxic to humans. However, Adt inhibitors which are not limited to the chloroplasts may also find utility in use as an herbicide.

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An identified compound which inhibits function of the wild-type AdT enzyme is utilized as an active ingredient in an herbicide. The active ingredient is normally applied in the form of compositions together with one or more agriculturally acceptable carriers, and can be applied to the crop area or plant to be treated, simultaneously or in succession, with further compounds. These additional compounds can include fertilizers, other herbicides, fungicides, bactericides, nematocides, or mixtures of several of these preparations, together with further carriers, surfactants or application-promoting adjuvants. The herbicide may be applied as a seed coating, a ground spray, incorporated into the soil, or applied directly to the plant. Preferably, the active ingredient of the present invention or an agrochemical composition which contains at least one of the active ingredients of the present invention are applied as a leaf preparation. Methods of herbicide preparation and application are well known to one skilled in the art.

Resistant mutants to the AdT-inhibiting compound can be identified by mutagenizing cells or organisms and growing the mutagenized populations in the presence of a concentration of the inhibitor sufficient to inhibit growth of the wild-type cells or organisms, and selecting cells or organisms from the populations that are able to grow more rapidly than wild-type cells or organisms. Mutagenesis can be accomplished by any one of the means well known to one skilled in the art, including: chemical mutagenesis (e.g., ethyl methanesulfonate); ultraviolet radiation; X-ray

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exposure; and gamma radiation (see, *e.g.*, Watson *et al.*, *Recombinant DNA, Second Edition* (1992) Chapter 11:191-211; Freifelder, *Molecular Biology* (1987) Chapter 11:293-313). The mutant individuals which have the ability to tolerate or resist the normally toxic levels of the inhibitor are genetically purified, the gene encoding the mutant AdT is isolated, and the DNA sequence of the mutant gene is determined and translated into a predicted amino acid sequence. The amino acids which differ between the wild-type AdT enzyme and the mutant AdT enzyme are assumed to be responsible for the inhibitor-resistant phenotype of the newly-identified mutant.

The coding DNA sequence for the mutant AdT can be introduced into the plant cell in a number of different ways that are well known to those of skill in the art. Examples of such methods include micro injection, electroporation, *Agrobacterium*-mediated transformation, direct gene transfer, and micro projectile bombardment. Techniques for producing herbicide resistance in plants by incorporating DNA encoding and expressing enzymes resistant to herbicides are well known (see, *e.g.*, U.S. Patent No. 5,145,777; U.S. Patent No. 5,290,926), including techniques for adding a chloroplast transit sequence upstream from an herbicide gene so that the protein product is transported into the chloroplasts (Comai *et al.*, *Nature* (1985) 313:741-744; U.S. Patent No. 4,940,835; U.S. Patent No. 5,188,642). In the same manner, the gene coding for a mutant AdT may be substituted for one of the other herbicide resistance genes of the references. Since AdT performs its function in the

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chloroplast, it may be particularly relevant to use a plastid transit sequence to ensure expression in the chloroplast or other plastid as is known in the art.

Following introduction of the mutant AdT gene into plant cells and the regeneration of transformed plants from such cells, conventional methods of plant husbandry and plant breeding can be used to maintain and increase the transformed plants. The transformed plants can also be used in conventional hybridization schemes to produce new plant types which also carry the novel mutant AdT gene (see, *e.g.*, Fehr and Hadley, *Hybridization of Crop Plants* (1980); Jensen, *Plant Breeding Methodology* (1988); Allard, *Principles of Plant Breeding* (1960).

Plants which express a gene which is tolerant or resistant to an inhibitor of AdT can be grown in soil and the herbicide containing the AdT inhibitor can be applied to inhibit weed growth. Since the weed plants will not be carrying the mutant AdT gene, the weeds will be susceptible to the herbicide containing the AdT inhibitor.

The following examples are intended to illustrate, but not to limit, aspects of the present invention.

EXAMPLES

The invention will be further described by reference to the following detailed examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified.

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Experimental Procedures

Preparation and purification of recombinant *Bacillus subtilis* Gln⁺RNA^{Gln} amidotransferase. *E. coli* BL21 (DE3) harboring pABC were incubated overnight in 3 mL LB medium (10 g bactotryptone, 5 g yeast extract, 10 g NaCl) with 50 (g/mL

5 ampicillin at 37°C. The culture was scaled up to 1 L and again allowed to incubate at 37°C overnight. Cells were harvested via centrifugation (4000 x g for 5 minutes at 4°C) and resuspended in 20 mL Buffer A (25 mM Hepes-KOH, pH 7.5, 25 mM KCl, 10 mM MgCl₂ and 1 mM DTT). This step and all subsequent steps were performed at 4°C unless otherwise specified. The cells were lysed by sonication (4 x 15

10 seconds) and centrifuged at 100,000 x g for one hour. The enzyme was then purified to homogeneity, as determined by SDS-polyacrylamide gel electrophoresis, via a series of chromatographic steps using a Pharmacia FPLC system. The supernatant was first applied to a Q-sepharose (HR 16/10) column (strong anion exchange) and the activity was eluted by a linear gradient from 0 to 1 M NaCl in Buffer A. The active

15 fractions from this column were applied to a Superdex-200 (HR 26/100) column (gel filtration) and the activity was eluted isocratically in Buffer A. The fractions from this column which contained activity were pooled and applied onto a MonoQ (HR 10/10) column and the activity as eluted with a linear gradient from 150 to 300 mM NaCl in Buffer A. Active fractions from this column were pooled and dialyzed

20 against Buffer A + 200 mM NaCl in 50% glycerol for 12 hours and stored at 70°C.

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In vivo expressed *Bacillus subtilis* tRNA^{Gln} isolation and purification. A

3 mL culture of *E. coli* DH5a/pGP1-2/pBTT (encoding tRNA^{Gln}) in LB medium (10 g bactotryptone, 5 g yeast extract, 10 g NaCl) with 50 (g/mL ampicillin and 10 (g/mL kanamycin was incubated at 37°C overnight. The culture was scaled up to
5 1 L and overnight incubation was repeated. Cells were harvested via centrifugation at 4000 x g for 5 minutes at 4°C and resuspended in 10 mL lysis buffer (20 mM Tris·HCl, pH 7.4 and 20 mM MgCl₂). Total nucleic acids were isolated by two sequential extractions with equal volumes of water saturated phenol followed by isopropanol precipitation of the aqueous phase. The nucleic acid pellet was collected
10 via centrifugation at 10,000 x g for 15 minutes at 4°C. The pellet was resuspended in 5 mL of 200 mM Tris·OAc, pH 9.0 and incubated at 37°C for 1 hour to ensure complete deacylation of the tRNA. The nucleic acids were recovered by ethanol precipitation followed by centrifugation at 10,000 x g for 15 minutes at 4°C. The pellet was resuspended in 100 mM NaCl, incubated overnight at 4°C and ethanol
15 precipitated. The tRNA^{Gln} was purified by a two-step anion exchange chromatography protocol. The nucleic acids were resuspended in 5 mL of Buffer 1 (140 mM NaOAc, pH 4.5) and 1 gm DE52 resin/100 OD260 was added. The resin was washed with 200 mL Buffer A and 150 mL Buffer 2 (140 mM NaOAc, pH 4.5 + 300 mM NaCl) and the tRNA was eluted with 100 mL Buffer 3 (140 mM NaOAc,
20 pH 4.5 + 1 M NaCl). the nucleic acids were recovered by ethanol precipitation

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followed by centrifugation at 10,000 x g for 15 minutes at 4°C and resuspended in 10 mM Tris·HCl, pH 7.4, 1 mM MgCl₂ and 1 mM DTT and applied onto a Pharmacia MonoQ (HR 10/10) column. The tRNA was eluted with a gradient of 450 to 750 mM NaCl in 10 mM Tris·HCl, pH 7.4, 1 mM MgCl₂ and 1 mM DTT. Fractions
5 containing the tRNA^{Gln}, based on ability to be aminoacylated with both Glu and Gln, were pooled and used as substrates in the amidotransferase assays.

Aminoacylation reactions. The procedure for the formation of radiolabelled Gln-tRNA^{Gln} was adapted from Jahn, D. et al. (1990). Unless otherwise noted, these reactions were conducted at 37°C in a buffer consisting of 10 mM ATP, 50 mM
10 Hepes·KOH pH 7.0, 25 mM KCl, 15 mM MgCl₂, and 5 mM DTT. The concentration of tRNA^{Gln}, recovered from *E. coli* DH5a harboring the plasmids pGP12 and pBTT (see above), and L14C(U)-glutamate (300 mCi/mMol) was 10 (M. GluRS was isolated from *B. subtilis* and then partially purified by DEAE-sepharose chromatography. The reactions were allowed to progress for various lengths of time
15 depending upon the assay. Aliquots from this mixture were then added to the amidotransferase assay mixtures either directly or following water saturated phenol extraction, ethanol precipitation, and resuspension in the aminoacylation buffer.

Amidotransferase reactions. The procedure for the formation of radiolabelled Gln-tRNA^{Gln} from Gln-tRNA^{Gln} was adapted from Jahn, D. et al. (1990).
20 Unless otherwise noted, these reactions were conducted at 37°C in a buffer consisting

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of 1 mM ATP, 5 mM Hepes·KOH pH 7.0, 2.5 mM KCl, 1.5 mM MgCl₂, and 0.5 mM dithiothreitol (DTT). The concentration of L14C(U)-GluRNA^{Gln} was 1 (M and the concentration of Lglutamine was 1 mM. Aliquots (0 to 20 (L) from fractions obtained during purification of the enzyme were added and the mixture was incubated for

5 various lengths of time depending upon the assay followed by quenching with 10 (L, 3 M NaOAc, pH 5.0. The mixture was extracted with an equal volume of water-saturated phenol and the aqueous and organic phases were separated by centrifugation at 15,000 x g at room temperature for 60 seconds. The aqueous phase was removed, 3x volumes of ethanol were added and the tRNA was precipitated at

10 70°C for 15 minutes. The precipitated tRNA was recovered by centrifugation at 15,000 x g at 4°C for 15 minutes. The pellet obtained was resuspended in 50 (L 0.01 N KOH and deaminoacylated at 65°C for 10 minutes. The base was neutralized with 1.3 (L, 0.1 N HCl (to pH (6 to 7) and the solution was dried completely under vacuum. The dried pellet was resuspended in 3 (L double-distilled H₂O and spotted

15 onto a TLC place (cellulose, Aldrich). The front was allowed to migrate 3.5 to 5 hours in one of two solvent systems (A. 20:1:5 isopropyl alcohol:formic acid:water or B. 2:1:6:6 ammonia:water:chloroform:methanol). The plate was dried at 85°C, exposed to an activated phosphoroimaging plate ((12 hours) and the image was analyzed using MacBas v2.0. In this way, the conversion of Glu to Gln was

20 measured.

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Example 1**Characterization of DNA Fragment Encoding AdT**

Three genes; one transcript of correct size hybridizing with three probes is provided in Figure 3 (SEQ ID NO:1). Open reading frames for each of the subunits is
5 provided.

Example 2**Characterization of *B. subtilis* AdT Protein**

The amino acid sequence of *B. subtilis* AdT is encoded by the nucleotide sequence of Figure 3 (SEQ ID NO:1).

10 The molecular weights of the three subunits is computed to be: 53.039 Kd, A subunit; 53.314 Kd, B subunit; and 10.859 Kd, C subunit. The amino acid sequences of each of the subunits A, B and C are provided in SEQ ID NOs: 4, 6 and 8, respectively.

The sizes of the subunits were confirmed via polyacrylamide gel
15 electrophoresis.

Example 3**Preparation of polyclonal antiserum containing anti-AdT antibodies**

A polyclonal antiserum containing anti-AdT antibodies was obtained by administered to rabbits recombinant AdT (trimeric protein) to rabbits following

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known methods. Incubation of the antiserum with a *B. subtilis* extract containing AdT protein completely inhibited AdT activity.

Using polyacrylamide gel electrophoresis, the antiserum was shown to contain antibodies immunoreactive to the assembled AdT protein. The polyclonal antisera

5 was significantly less immunoreactive to non-assembled, individual subunits.

Example 4

Production and purification of AdT

10

| Table I. Activity of cell extracts from <i>E. coli</i> BL21(DE3) harboring various vectors. | | |
|---------------------------------------------------------------------------------------------|-------------------------------------|-------------------|
| Vector | Glutamine Recovered <i>pMole</i> | Relative Activity |
| pABC | 2.03 ± 0.28 | 1.000 |
| pA | 0.02 ± 0.02 | 0.010 |
| pB | 0.03 ± 0.01 | 0.015 |
| 15 pC | 0.03 ± 0.02 | 0.017 |

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Table II. Purification of *Bacillus subtilis* GlnTRNA^{Gln} amidotransferase over expressed in *Escherichia coli* BL21(DE3).

| Purification Step | Total volume mL | Total protein mg | Total activity ^a units x 10 ³ | Specific activity units/mg | Yield % | -fold |
|-------------------|--------------------|---------------------|--------------------------------------------------------|-------------------------------|------------|-------|
| S-100 | 15 | 190 | 13.5 | 70 | 100 | 1 |
| Q-sepharoseFF | 12 | 40 | 8.1 | 200 | 60 | 3 |
| Superdex-200 | 10 | 4.6 | 13.4 | 3000 | 99 | 40 |
| MonoQ | 3.5 | 1.0 | 8.3 | 9000 | 61 | 130 |

^a One unit is defined as 1 pMole glutamine produced per minute at 37°C under the assay conditions described in materials and methods.

Example 5

Identifying inhibitors of AdT activity

Purified amidotransferase is used in an assay to identify inhibitors of AdT

activity. The assay used to identify inhibitors of AdT activity comprises:

- (a) incubating a first sample of AdT and its substrate;
- (b) measuring an uninhibited reactivity of the AdT from step (a);
- (c) incubating a first sample of AdT and its substrate in the presence of a second sample comprising an inhibitor compound;
- (d) measuring an inhibited reactivity of the AdT from step (c); and,

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(e) comparing the inhibited reactivity to the uninhibited reactivity of the AdT.

Inhibitors of AdT identified using this process are utilized as antibacterial, antifungal and herbicidal agents.

5

Example 6

Identification of inhibitor-resistant AdT mutants

Purified amidotransferase and an identified inhibitor of AdT is used in an assay to identify inhibitor-resistant AdT mutants. The assay used to identify inhibitor-resistant AdT mutants comprises :

- 10 (a) incubating a first sample of AdT and its substrate in the presence of a second sample comprising an AdT inhibitor;
- (b) measuring an unmutated reactivity of the AdT from step (a);
- (c) incubating a first sample of a mutated AdT and its substrate in the presence of a second sample comprising an AdT inhibitor;
- 15 (d) measuring a mutated reactivity of the mutated AdT from step (c); and,
- (e) comparing the mutated reactivity to the unmutated reactivity of the AdT.

Inhibitor-resistant AdT mutants identified using this process are utilized in the production of cells and organisms resistant to AdT inhibitors.

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Example 7**Diagnostic assays**

Nucleic acids for diagnosis are obtained from cells or tissues. Genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. The genomic DNA can be compared to the polynucleotide coding for amidotransferase as provided in SEQ ID NO:1. Deletions and insertions can be detected by a change in size of the amplified product in comparison to SEQ ID NO:1. Point mutations can be identified by hybridizing amplified DNA to labeled AdT polynucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNASE digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in the electrophoretic mobility of the DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase and S1 protection or a chemical cleavage method.

Cells carrying mutations or polymorphisms in the gene of the invention may also be detected at the DNA level by a variety of techniques. For example, RTPCR can be used to detect mutations. It is particularly preferred to use RTPCR in conjunction with automated detection systems, such as, for example, GeneScan. RNA or cDNA may also be used for the same purpose, PCR or RTPCR. As an example, PCR primers complementary to the nucleic acid encoding AdT can be used to identify

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and analyze mutations. These primers may be used for amplifying AdT DNA isolated from a sample derived from an organism. The invention also provides these primers with 1, 2, 3 or 4 nucleotides removed from the 5' and/or the 3' end. The primers may be used to amplify the gene isolated from an infected individual such that the gene
5 may then be subject to various techniques for elucidation of the DNA sequence. In this way, mutations in the AdT DNA sequence may be detected and used to diagnose infection and to serotype or classify the infectious agent.

Increased or decreased expression of AdT polynucleotide can be measured using any of the methods well known in the art for the quantitation of polynucleotides,
10 such as, for example, amplification, PCR, RTPCR, RNase protection, Northern blotting and other hybridization methods.

In addition, a diagnostic assay in accordance with the intention for detecting over-or under- expression of AdT protein compared to normal control tissue samples. Assay techniques that can be used to determine levels of AdT protein, in a sample
15 derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassay, competitive-binding assays, Western Blot analysis and ELISA assays.

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Example 8

Production of transformed plants

5 A mutated AdT-encoding DNA sequence that confers resistance to AdT inhibitors is isolated from an inhibitor-resistant AdT mutant using mutagenesis and isolation techniques well known to one of skill in the art. The coding sequence for the mutant AdT gene is then introduced into a plant cell and whole transformed plants are regenerated from the transformed plant cell using a number of different techniques
10 well known to those of skill in the art. The transformed plants are used in conventional plant breeding schemes to produce new varieties of plants which also carry the mutant AdT gene. Crop plants carrying the mutant AdT gene are grown in production and an herbicide comprising an AdT inhibitor is applied to the crop to control weeds.

15 Although the present invention has been described in detail with reference to examples above, it is understood that various modifications can be made without departing from the spirit of the invention. All cited references referred to in the application are hereby incorporated by reference.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Soll, Dieter
- (ii) TITLE OF INVENTION: GLU-TRAN AMIDOTRANSFERASE - A NOVEL
ESSENTIAL TRANSLATIONAL COMPONENT
- (iii) NUMBER OF SEQUENCES: 8
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: MORGAN, LEWIS & BOCKIUS LLP
 - (B) STREET: 1800 M Street, N.W.
 - (C) CITY: Washington
 - (D) STATE: D.C.
 - (E) COUNTRY: USA
 - (F) ZIP: 20036-5869
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US Unassigned
 - (B) FILING DATE: 03-FEB-1998
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 60/037,275
 - (B) FILING DATE: 03-FEB-1997
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Adler, Reid G.
 - (B) REGISTRATION NUMBER: 30,988
 - (C) REFERENCE/DOCKET NUMBER: 044574-5024-WO
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 202-467-7000
 - (B) TELEFAX: 202-467-7176

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3495 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: join(1..54, 58..390, 394..1866, 1870..3303, 3310
..3321, 3325..3348, 3352..3429, 3433..3471, 3475
..3480, 3484..3495)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

| | |
|-----------------------------------------------------------------|-----|
| GAA TTC GAT CCT GTC TCA AGG CGT TTT GTT GCT TTA AAG GGC TTG TTT | 48 |
| Glu Phe Asp Pro Val Ser Arg Arg Phe Val Ala Leu Lys Gly Leu Phe | |
| 1 5 10 15 | |
| TTG ATA TGA TCA GTA TTA TAT GAC TTA ACG GAG AAA TAT GTG GAG GTG | 96 |
| Leu Ile Ser Val Leu Tyr Asp Leu Thr Glu Lys Tyr Val Glu Val | |
| 20 25 30 | |
| GAT CAT ATG TCA CGA ATT TCA ATA GAA GAA GTA AAG CAC GTT GCG CAC | 144 |
| Asp His Met Ser Arg Ile Ser Ile Glu Glu Val Lys His Val Ala His | |
| 35 40 45 | |
| CTT GCA AGA CTT GCG ATT ACT GAA GAA GAA GCA AAA ATG TTC ACT GAA | 192 |
| Leu Ala Arg Leu Ala Ile Thr Glu Glu Glu Ala Lys Met Phe Thr Glu | |
| 50 55 60 | |
| CAG CTC GAC AGT ATC ATT TCA TTT GCC GAG GAG CTT AAT GAG GTT AAC | 240 |
| Gln Leu Asp Ser Ile Ile Ser Phe Ala Glu Glu Leu Asn Glu Val Asn | |
| 65 70 75 | |
| ACA GAC AAT GTG GAG CCT ACA ACT CAC GTG CTG AAA ATG AAA AAT GTC | 288 |
| Thr Asp Asn Val Glu Pro Thr Thr His Val Leu Lys Met Lys Asn Val | |
| 80 85 90 95 | |
| ATG AGA GAA GAT GAA GCG GGT AAA GGT CTT CCG GTT GAG GAT GTC ATG | 336 |
| Met Arg Glu Asp Glu Ala Gly Lys Gly Leu Pro Val Glu Asp Val Met | |
| 100 105 110 | |
| AAA AAT GCG CCT GAC CAT AAA GAC GGC TAT ATT CGT GTG CCA TCA ATT | 384 |
| Lys Asn Ala Pro Asp His Lys Asp Gly Tyr Ile Arg Val Pro Ser Ile | |
| 115 120 125 | |
| CTG GAC TAA AGG AGG GAC ACA AGA ATG TCA TTA TTT GAT CAT AAA ATC | 432 |
| Leu Asp Arg Arg Asp Thr Arg Met Ser Leu Phe Asp His Lys Ile | |
| 130 135 140 | |
| ACA GAA TTA AAA CAG CTC ATA CAT AAA AAA GAG ATT AAG ATT TCT GAT | 480 |
| Thr Glu Leu Lys Gln Leu Ile His Lys Lys Glu Ile Lys Ile Ser Asp | |
| 145 150 155 | |
| CTG GTT GAT GAA TCT TAT AAA CGC ATC CAA GCG GTT GAT GAT AAG GTA | 528 |
| Leu Val Asp Glu Ser Tyr Lys Arg Ile Gln Ala Val Asp Asp Lys Val | |
| 160 165 170 | |
| CAA GCC TTT TTG GCA TTA GAT GAA GAA AGA GCG CGC GCA TAC GCG AAG | 576 |
| Gln Ala Phe Leu Ala Leu Asp Glu Glu Arg Ala Arg Ala Tyr Ala Lys | |
| 175 180 185 190 | |
| GAG CTT GAT GAG GCG GTT GAC GGC CGT TCT GAG CAC GGT CTT CTT TTC | 624 |
| Glu Leu Asp Glu Ala Val Asp Gly Arg Ser Glu His Gly Leu Leu Phe | |
| 195 200 205 | |
| GGT ATG CCG ATC GGC GTA AAA GAT AAT ATC GTA ACA AAA GGG CTG CGC | 672 |
| Gly Met Pro Ile Gly Val Lys Asp Asn Ile Val Thr Lys Gly Leu Arg | |
| 210 215 220 | |

| | |
|-----------------------------------------------------------------|------|
| ACA ACA TGC TCC AGC AAA ATT CTC GAA AAC TTT GAT CCG ATT TAC GAT | 720 |
| Thr Thr Cys Ser Ser Lys Ile Leu Glu Asn Phe Asp Pro Ile Tyr Asp | |
| 225 230 235 | |
| GCT ACT GTC GTT CAG CGC CTT CAA GAC GCT GAA GCG GTC ACA ATC GGA | 768 |
| Ala Thr Val Val Gln Arg Leu Gln Asp Ala Glu Ala Val Thr Ile Gly | |
| 240 245 250 | |
| AAA CTG AAC ATG GAC GAA TTC GCC ATG GGG TCA TCT ACA GAA AAC TCA | 816 |
| Lys Leu Asn Met Asp Glu Phe Ala Met Gly Ser Ser Thr Glu Asn Ser | |
| 255 260 265 270 | |
| GCT TAC AAG CTG ACG AAA AAC CCT TGG AAC CTG GAT ACA GTT CCC GGC | 864 |
| Ala Tyr Lys Leu Thr Lys Asn Pro Trp Asn Leu Asp Thr Val Pro Gly | |
| 275 280 285 | |
| GGT TCA AGC GGC GGA TCT GCA GCT GCG GTT GCT GCG GGA GAA GTT CCG | 912 |
| Gly Ser Ser Gly Gly Ser Ala Ala Val Ala Ala Gly Glu Val Pro | |
| 290 295 300 | |
| TTT TCT CTT GGA TCT GAC ACA GGC GGC TCC ATC CGT CAG CCG GCA TCT | 960 |
| Phe Ser Leu Gly Ser Asp Thr Gly Gly Ser Ile Arg Gln Pro Ala Ser | |
| 305 310 315 | |
| TTC TGC GGC GTT GTC GGA TTA AAA CCT ACA TAC GGA CGT GTA TCT CGT | 1008 |
| Phe Cys Gly Val Val Gly Leu Lys Pro Thr Tyr Gly Arg Val Ser Arg | |
| 320 325 330 | |
| TAC GGC CTG GTC GCA TTT GCG TCT TCA TTG GAC CAA ATC GGA CCG ATT | 1056 |
| Tyr Gly Leu Val Ala Phe Ala Ser Ser Leu Asp Gln Ile Gly Pro Ile | |
| 335 340 345 350 | |
| ACA CGT ACG GTT GAG GAT AAC GCG TTT TTA CTT CAA GCG ATT TCC GGC | 1104 |
| Thr Arg Thr Val Glu Asp Asn Ala Phe Leu Leu Gln Ala Ile Ser Gly | |
| 355 360 365 | |
| GTA GAC AAA ATG GAC TCT ACG AGT GCA AAT GTG GAC GTG CCT GAT TTT | 1152 |
| Val Asp Lys Met Asp Ser Thr Ser Ala Asn Val Asp Val Pro Asp Phe | |
| 370 375 380 | |
| CTT TCT TCA TTA ACT GGC GAC ATC AAA GGA CTG AAA ATC GCC GTT CCG | 1200 |
| Leu Ser Ser Leu Thr Gly Asp Ile Lys Gly Leu Lys Ile Ala Val Pro | |
| 385 390 395 | |
| AAA GAA TAC CTT GGT GAA GGT GTC GGC AAA GAA GCG AGA GAA TCT GTC | 1248 |
| Lys Glu Tyr Leu Gly Glu Gly Val Gly Lys Glu Ala Arg Glu Ser Val | |
| 400 405 410 | |
| TTG GCA GCG CTG AAA GTC CTT GAA GGT CTC GGC GCT ACA TGG GAA GAA | 1296 |
| Leu Ala Ala Leu Lys Val Leu Glu Gly Leu Gly Ala Thr Trp Glu Glu | |
| 415 420 425 430 | |
| GTG TCT CTT CCG CAC AGT AAA TAC GCG CTT GCG ACA TAT TAC CTG CTG | 1344 |
| Val Ser Leu Pro His Ser Lys Tyr Ala Leu Ala Thr Tyr Tyr Leu Leu | |
| 435 440 445 | |
| TCA TCT TCT GAA GCG TCA GCG AAC CTT GCA CGC TTT GAC GGC ATC CGC | 1392 |
| Ser Ser Ser Glu Ala Ser Ala Asn Leu Ala Arg Phe Asp Gly Ile Arg | |
| 450 455 460 | |

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| | |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| TAC GGC TAC CGC ACA GAC AAC GCG GAT AAC CTG ATC GAC CTT TAC AAG Tyr Gly Tyr Arg Thr Asp Asn Ala Asp Asn Leu Ile Asp Leu Tyr Lys 465 470 475 | 1440 |
| CAA ACG CGC GCT GAA GGT TTC GGA AAT GAA GTC AAA CGC CGC ATC ATG Gln Thr Arg Ala Glu Gly Phe Gly Asn Glu Val Lys Arg Arg Ile Met 480 485 490 | 1488 |
| CTC GGA ACG TTT GCT TTA AGC TCA GGC TAC TAC GAT GCG TAC TAC AAA Leu Gly Thr Phe Ala Leu Ser Ser Gly Tyr Tyr Asp Ala Tyr Tyr Lys 495 500 505 510 | 1536 |
| AAA GCG CAA AAA GTG CGT ACG TTG ATT AAG AAG GAT TTC GAG GAC GTA Lys Ala Gln Lys Val Arg Thr Leu Ile Lys Lys Asp Phe Glu Asp Val 515 520 525 | 1584 |
| TTT GAA AAA TAT GAT GTT ATT GTT GGA CCG ACT ACA CCG ACA CCT GCG Phe Glu Lys Tyr Asp Val Ile Val Gly Pro Thr Thr Pro Thr Pro Ala 530 535 540 | 1632 |
| TTT AAA ATC GGT GAA AAC ACG AAG GAT CCG CTC ACA ATG TAC GCA AAC Phe Lys Ile Gly Glu Asn Thr Lys Asp Pro Leu Thr Met Tyr Ala Asn 545 550 555 | 1680 |
| GAT ATC TTA ACG ATT CCG GTC AAC CTT GCG GCG TAC CGG GAA TCA GGT Asp Ile Leu Thr Ile Pro Val Asn Leu Ala Ala Tyr Arg Glu Ser Gly 560 565 570 | 1728 |
| GCC ATG CGG TTA GCA GAC GGA CTT CCG CTC GGC CTG CAA ATC ATC GGA Ala Met Arg Leu Ala Asp Gly Leu Pro Leu Gly Leu Gln Ile Ile Gly 575 580 585 590 | 1776 |
| AAA CAC TTT GAT GAA GCA CTG TAT ACC GCG TTG CTC ATG CAT TTG AAC Lys His Phe Asp Glu Ala Leu Tyr Thr Ala Leu Leu Met His Leu Asn 595 600 605 | 1824 |
| AAG CAA CAG ACC ATC ATA AAG CAA AAC CTG AAC TGT AAG GGG Lys Gln Gln Thr Ile Ile Lys Gln Asn Leu Asn Cys Lys Gly 610 615 620 | 1866 |
| TGA AAA GAA TTG AAC TTT GAA ACG GTA ATC GGA CTT GAA GTC CAC GTT Lys Glu Leu Asn Phe Glu Thr Val Ile Gly Leu Glu Val His Val 625 630 635 | 1914 |
| GAG TTA AAA ACA AAA TCA AAA ATT TTC TCA AGC TCT CCA ACG CCA TTC Glu Leu Lys Thr Lys Ser Lys Ile Phe Ser Ser Ser Pro Thr Pro Phe 640 645 650 | 1962 |
| GGC GCG GAG GCG AAT ACG CAG ACA AGC GTT ATT GAC CTC GGA TAT CCG Gly Ala Glu Ala Asn Thr Gln Thr Ser Val Ile Asp Leu Gly Tyr Pro 655 660 665 | 2010 |
| GGC GTC CTG CCT GTT CTG AAC AAA GAA GCC GTT GAA TTC GCA ATG AAA Gly Val Leu Pro Val Leu Asn Lys Glu Ala Val Glu Phe Ala Met Lys 670 675 680 | 2058 |
| GCC GCT ATG GCG CTC AAC TGT GAG ATC GCA ACG GAT ACG AAG TTT GAC Ala Ala Met Ala Leu Asn Cys Glu Ile Ala Thr Asp Thr Lys Phe Asp 685 690 695 | 2106 |

| | |
|-----------------------------------------------------------------|------|
| CGC AAA AAC TAT TTC TAT CCT GAC AAC CCG AAA GCG TAT CAG ATT TCT | 2154 |
| Arg Lys Asn Tyr Phe Tyr Pro Asp Asn Pro Lys Ala Tyr Gln Ile Ser | |
| 700 705 710 715 | |
| CAA TTT GAT AAG CCA ATC GGC GAA AAC GGC TGG ATC GAA ATT GAA GTC | 2202 |
| Gln Phe Asp Lys Pro Ile Gly Glu Asn Gly Trp Ile Glu Ile Glu Val | |
| 720 725 730 | |
| GGC GGC AAA ACA AAA CGC ATC GGC ATC ACG CGC CTT CAT CTT GAA GAG | 2250 |
| Gly Gly Lys Thr Lys Arg Ile Gly Ile Thr Arg Leu His Leu Glu Glu | |
| 735 740 745 | |
| GAT GCC GGA AAA CTG ACG CAT ACG GGC GAC GGC TAT TCT CTT GTT GAC | 2298 |
| Asp Ala Gly Lys Leu Thr His Thr Gly Asp Gly Tyr Ser Leu Val Asp | |
| 750 755 760 | |
| TTC AAC CGT CAA GGA ACG CCG CTT GTT GAG TNC GTA TCA GAG CCG GAC | 2346 |
| Phe Asn Arg Gln Gly Thr Pro Leu Val Glu Xaa Val Ser Glu Pro Asp | |
| 765 770 775 | |
| ATC CGC ACG CCG GAA GAA NCG TAC GCA TAT CTT GAA AAG CTG AAA TCC | 2394 |
| Ile Arg Thr Pro Glu Glu Xaa Tyr Ala Tyr Leu Glu Lys Leu Lys Ser | |
| 780 785 790 795 | |
| ATC ATC CAA TAT ACA GGC GTT TCT GAC TGT AAA ATG GAA GAA GGC TCA | 2442 |
| Ile Ile Gln Tyr Thr Gly Val Ser Asp Cys Lys Met Glu Glu Gly Ser | |
| 800 805 810 | |
| CTT CGC TGT GAC GCC AAT ATC TCT CTT CGT CCG ATC GGC CAA GAG GAA | 2490 |
| Leu Arg Cys Asp Ala Asn Ile Ser Leu Arg Pro Ile Gly Gln Glu Glu | |
| 815 820 825 | |
| TTC GGC ACA AAA ACA GAA TTG AAA AAC TTG AAC TCC TTT GCG TTT GTT | 2538 |
| Phe Gly Thr Lys Thr Glu Leu Lys Asn Leu Asn Ser Phe Ala Phe Val | |
| 830 835 840 | |
| CAA AAA GGC CTT GAG CAT GAA GAA AAA CGC CAG GAG CAG GTT CTT CTT | 2586 |
| Gln Lys Gly Leu Glu His Glu Glu Lys Arg Gln Glu Gln Val Leu Leu | |
| 845 850 855 | |
| TCC GGC TTC TTC ATC CAG CAA GAA ACT CGC CGT TAT GAT GAA GCA ACG | 2634 |
| Ser Gly Phe Phe Ile Gln Gln Glu Thr Arg Arg Tyr Asp Glu Ala Thr | |
| 860 865 870 875 | |
| AAG AAA ACC ATT CTT ATG CGT GTC AAA GAG GGA TCT GAC GAC TAC CGT | 2682 |
| Lys Lys Thr Ile Leu Met Arg Val Lys Glu Gly Ser Asp Asp Tyr Arg | |
| 880 885 890 | |
| TAC TTC CCA GAG CCA GAT CTA GTC GAG CTC TAC ATT GAT GAT GAA TGG | 2730 |
| Tyr Phe Pro Glu Pro Asp Leu Val Glu Leu Tyr Ile Asp Asp Glu Trp | |
| 895 900 905 | |
| AAG GAA CGC GTA AAA GCA AGC ATT CCT GAG CTT CCG GAT GAG CGC CGC | 2778 |
| Lys Glu Arg Val Lys Ala Ser Ile Pro Glu Leu Pro Asp Glu Arg Arg | |
| 910 915 920 | |
| AAG CGT TAT ATC GAA GAG CTT GGC TTC GCT GCA TAT GAC GCA ATG GTT | 2826 |
| Lys Arg Tyr Ile Glu Glu Leu Gly Phe Ala Ala Tyr Asp Ala Met Val | |
| 925 930 935 | |

| | |
|-----------------------------------------------------------------|------|
| CTG ACG CTG ACA AAA GAA ATG GCT GAT TTC TTC GAA GAA ACC GTT CAA | 2874 |
| Leu Thr Leu Thr Lys Glu Met Ala Asp Phe Phe Glu Glu Thr Val Gln | |
| 940 945 950 955 | |
| AAA GGC GCT GAA GCT AAA CAA GCG TCT AAC TGG CTG ATG GGT GAA GTG | 2922 |
| Lys Gly Ala Glu Ala Lys Gln Ala Ser Asn Trp Leu Met Gly Glu Val | |
| 960 965 970 | |
| TCA GCT TAC CTA AAC GCA GAA CAA AAA GAG CTT GCC GAT GTT GCC CTG | 2970 |
| Ser Ala Tyr Leu Asn Ala Glu Gln Lys Glu Leu Ala Asp Val Ala Leu | |
| 975 980 985 | |
| ACA CCT GAA GGC CTT GCC GGC ATG ATC AAA TTG ATT GAA AAA GGA ACC | 3018 |
| Thr Pro Glu Gly Leu Ala Gly Met Ile Lys Leu Ile Glu Lys Gly Thr | |
| 990 995 1000 | |
| ATT TCT TCT AAG ATC GCG AAG AAA GTG TTT AAA GAA TTG ATT GAA AAA | 3066 |
| Ile Ser Ser Lys Ile Ala Lys Lys Val Phe Lys Glu Leu Ile Glu Lys | |
| 1005 1010 1015 | |
| GGC GGC GAC GCT GAG AAG ATT GTG AAA GAG AAA GGC CTT GTT CAG ATT | 3114 |
| Gly Gly Asp Ala Glu Lys Ile Val Lys Glu Lys Gly Leu Val Gln Ile | |
| 1020 1025 1030 1035 | |
| TCT GAC GAA GGC GTG CTT CTG AAG CTT GTC ACT GAG GCG CTT GAC AAC | 3162 |
| Ser Asp Glu Gly Val Leu Leu Lys Leu Val Thr Glu Ala Leu Asp Asn | |
| 1040 1045 1050 | |
| AAT CCT CAA TCA ATC GAA GAC TTT AAA AAC GGA AAA GAC CGC GCG ATC | 3210 |
| Asn Pro Gln Ser Ile Glu Asp Phe Lys Asn Gly Lys Asp Arg Ala Ile | |
| 1055 1060 1065 | |
| GGC TTC CTA GTC GGA CAG ATT ATG AAA GCG TCC AAA GGA CAA GCC AAC | 3258 |
| Gly Phe Leu Val Gly Gln Ile Met Lys Ala Ser Lys Gly Gln Ala Asn | |
| 1070 1075 1080 | |
| CCG CCG ATG GTC AAC AAA ATT CTG CTT GAA GAA ATT AAA AAA CGC | 3303 |
| Pro Pro Met Val Asn Lys Ile Leu Leu Glu Glu Ile Lys Lys Arg | |
| 1085 1090 1095 | |
| TAATAA AAA AGC AGC CCT TAG AGG CTG CTT TTT TTA TGG TCA AAT | 3348 |
| Lys Ser Ser Pro Arg Leu Leu Phe Leu Trp Ser Asn | |
| 1100 1105 1110 | |
| TGA GAT AAA GAC AAG ATG AGG GCC CGA AGC CTT TCA ACT TCT TTG TCG | 3396 |
| Asp Lys Asp Lys Met Arg Ala Arg Ser Leu Ser Thr Ser Leu Ser | |
| 1115 1120 1125 | |
| TTG GTT CCG GCC AAA TTG GAC AGC ATG CCT TTA TAA TCG GCT TGC GCG | 3444 |
| Leu Val Pro Ala Lys Leu Asp Ser Met Pro Leu Ser Ala Cys Ala | |
| 1130 1135 1140 | |
| GTT TAT CCT GAG TCA ATT CTT CCT CGA TAA GAT AAG TGA CAC GGT GAT | 3492 |
| Val Tyr Pro Glu Ser Ile Leu Pro Arg Asp Lys His Gly Asp | |
| 1145 1150 | |
| ATC | 3495 |
| Ile | |
| 1155 | |

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1155 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Glu Phe Asp Pro Val Ser Arg Arg Phe Val Ala Leu Lys Gly Leu Phe
 1 5 10 15
 Leu Ile Ser Val Leu Tyr Asp Leu Thr Glu Lys Tyr Val Glu Val Asp
 20 25 30
 His Met Ser Arg Ile Ser Ile Glu Glu Val Lys His Val Ala His Leu
 35 40 45
 Ala Arg Leu Ala Ile Thr Glu Glu Glu Ala Lys Met Phe Thr Glu Gln
 50 55 60
 Leu Asp Ser Ile Ile Ser Phe Ala Glu Glu Leu Asn Glu Val Asn Thr
 65 70 75 80
 Asp Asn Val Glu Pro Thr Thr His Val Leu Lys Met Lys Asn Val Met
 85 90 95
 Arg Glu Asp Glu Ala Gly Lys Gly Leu Pro Val Glu Asp Val Met Lys
 100 105 110
 Asn Ala Pro Asp His Lys Asp Gly Tyr Ile Arg Val Pro Ser Ile Leu
 115 120 125
 Asp Arg Arg Asp Thr Arg Met Ser Leu Phe Asp His Lys Ile Thr Glu
 130 135 140
 Leu Lys Gln Leu Ile His Lys Lys Glu Ile Lys Ile Ser Asp Leu Val
 145 150 155 160
 Asp Glu Ser Tyr Lys Arg Ile Gln Ala Val Asp Asp Lys Val Gln Ala
 165 170 175
 Phe Leu Ala Leu Asp Glu Glu Arg Ala Arg Ala Tyr Ala Lys Glu Leu
 180 185 190
 Asp Glu Ala Val Asp Gly Arg Ser Glu His Gly Leu Leu Phe Gly Met
 195 200 205
 Pro Ile Gly Val Lys Asp Asn Ile Val Thr Lys Gly Leu Arg Thr Thr
 210 215 220
 Cys Ser Ser Lys Ile Leu Glu Asn Phe Asp Pro Ile Tyr Asp Ala Thr
 225 230 235 240
 Val Val Gln Arg Leu Gln Asp Ala Glu Ala Val Thr Ile Gly Lys Leu
 245 250 255
 Asn Met Asp Glu Phe Ala Met Gly Ser Ser Thr Glu Asn Ser Ala Tyr
 260 265 270

Lys Leu Thr Lys Asn Pro Trp Asn Leu Asp Thr Val Pro Gly Gly Ser
 275 280 285
 Ser Gly Gly Ser Ala Ala Ala Val Ala Ala Gly Glu Val Pro Phe Ser
 290 295 300
 Leu Gly Ser Asp Thr Gly Gly Ser Ile Arg Gln Pro Ala Ser Phe Cys
 305 310 315 320
 Gly Val Val Gly Leu Lys Pro Thr Tyr Gly Arg Val Ser Arg Tyr Gly
 325 330 335
 Leu Val Ala Phe Ala Ser Ser Leu Asp Gln Ile Gly Pro Ile Thr Arg
 340 345 350
 Thr Val Glu Asp Asn Ala Phe Leu Leu Gln Ala Ile Ser Gly Val Asp
 355 360 365
 Lys Met Asp Ser Thr Ser Ala Asn Val Asp Val Pro Asp Phe Leu Ser
 370 375 380
 Ser Leu Thr Gly Asp Ile Lys Gly Leu Lys Ile Ala Val Pro Lys Glu
 385 390 395 400
 Tyr Leu Gly Glu Gly Val Gly Lys Glu Ala Arg Glu Ser Val Leu Ala
 405 410 415
 Ala Leu Lys Val Leu Glu Gly Leu Gly Ala Thr Trp Glu Glu Val Ser
 420 425 430
 Leu Pro His Ser Lys Tyr Ala Leu Ala Thr Tyr Tyr Leu Leu Ser Ser
 435 440 445
 Ser Glu Ala Ser Ala Asn Leu Ala Arg Phe Asp Gly Ile Arg Tyr Gly
 450 455 460
 Tyr Arg Thr Asp Asn Ala Asp Asn Leu Ile Asp Leu Tyr Lys Gln Thr
 465 470 475 480
 Arg Ala Glu Gly Phe Gly Asn Glu Val Lys Arg Arg Ile Met Leu Gly
 485 490 495
 Thr Phe Ala Leu Ser Ser Gly Tyr Tyr Asp Ala Tyr Tyr Lys Lys Ala
 500 505 510
 Gln Lys Val Arg Thr Leu Ile Lys Lys Asp Phe Glu Asp Val Phe Glu
 515 520 525
 Lys Tyr Asp Val Ile Val Gly Pro Thr Thr Pro Thr Pro Ala Phe Lys
 530 535 540
 Ile Gly Glu Asn Thr Lys Asp Pro Leu Thr Met Tyr Ala Asn Asp Ile
 545 550 555 560
 Leu Thr Ile Pro Val Asn Leu Ala Ala Tyr Arg Glu Ser Gly Ala Met
 565 570 575
 Arg Leu Ala Asp Gly Leu Pro Leu Gly Leu Gln Ile Ile Gly Lys His
 580 585 590

Phe Asp Glu Ala Leu Tyr Thr Ala Leu Leu Met His Leu Asn Lys Gln
 595 600 605
 Gln Thr Ile Ile Lys Gln Asn Leu Asn Cys Lys Gly Lys Glu Leu Asn
 610 615 620
 Phe Glu Thr Val Ile Gly Leu Glu Val His Val Glu Leu Lys Thr Lys
 625 630 635 640
 Ser Lys Ile Phe Ser Ser Ser Pro Thr Pro Phe Gly Ala Glu Ala Asn
 645 650 655
 Thr Gln Thr Ser Val Ile Asp Leu Gly Tyr Pro Gly Val Leu Pro Val
 660 665 670
 Leu Asn Lys Glu Ala Val Glu Phe Ala Met Lys Ala Ala Met Ala Leu
 675 680 685
 Asn Cys Glu Ile Ala Thr Asp Thr Lys Phe Asp Arg Lys Asn Tyr Phe
 690 695 700
 Tyr Pro Asp Asn Pro Lys Ala Tyr Gln Ile Ser Gln Phe Asp Lys Pro
 705 710 715 720
 Ile Gly Glu Asn Gly Trp Ile Glu Ile Glu Val Gly Gly Lys Thr Lys
 725 730 735
 Arg Ile Gly Ile Thr Arg Leu His Leu Glu Glu Asp Ala Gly Lys Leu
 740 745 750
 Thr His Thr Gly Asp Gly Tyr Ser Leu Val Asp Phe Asn Arg Gln Gly
 755 760 765
 Thr Pro Leu Val Glu Xaa Val Ser Glu Pro Asp Ile Arg Thr Pro Glu
 770 775 780
 Glu Xaa Tyr Ala Tyr Leu Glu Lys Leu Lys Ser Ile Ile Gln Tyr Thr
 785 790 795 800
 Gly Val Ser Asp Cys Lys Met Glu Glu Gly Ser Leu Arg Cys Asp Ala
 805 810 815
 Asn Ile Ser Leu Arg Pro Ile Gly Gln Glu Glu Phe Gly Thr Lys Thr
 820 825 830
 Glu Leu Lys Asn Leu Asn Ser Phe Ala Phe Val Gln Lys Gly Leu Glu
 835 840 845
 His Glu Glu Lys Arg Gln Glu Gln Val Leu Leu Ser Gly Phe Phe Ile
 850 855 860
 Gln Gln Glu Thr Arg Arg Tyr Asp Glu Ala Thr Lys Lys Thr Ile Leu
 865 870 875 880
 Met Arg Val Lys Glu Gly Ser Asp Asp Tyr Arg Tyr Phe Pro Glu Pro
 885 890 895
 Asp Leu Val Glu Leu Tyr Ile Asp Asp Glu Trp Lys Glu Arg Val Lys
 900 905 910

Ala Ser Ile Pro Glu Leu Pro Asp Glu Arg Arg Lys Arg Tyr Ile Glu
 915 920 925
 Glu Leu Gly Phe Ala Ala Tyr Asp Ala Met Val Leu Thr Leu Thr Lys
 930 935 940
 Glu Met Ala Asp Phe Phe Glu Glu Thr Val Gln Lys Gly Ala Glu Ala
 945 950 955 960
 Lys Gln Ala Ser Asn Trp Leu Met Gly Glu Val Ser Ala Tyr Leu Asn
 965 970 975
 Ala Glu Gln Lys Glu Leu Ala Asp Val Ala Leu Thr Pro Glu Gly Leu
 980 985 990
 Ala Gly Met Ile Lys Leu Ile Glu Lys Gly Thr Ile Ser Ser Lys Ile
 995 1000 1005
 Ala Lys Lys Val Phe Lys Glu Leu Ile Glu Lys Gly Gly Asp Ala Glu
 1010 1015 1020
 Lys Ile Val Lys Glu Lys Gly Leu Val Gln Ile Ser Asp Glu Gly Val
 1025 1030 1035 1040
 Leu Leu Lys Leu Val Thr Glu Ala Leu Asp Asn Asn Pro Gln Ser Ile
 1045 1050 1055
 Glu Asp Phe Lys Asn Gly Lys Asp Arg Ala Ile Gly Phe Leu Val Gly
 1060 1065 1070
 Gln Ile Met Lys Ala Ser Lys Gly Gln Ala Asn Pro Pro Met Val Asn
 1075 1080 1085
 Lys Ile Leu Leu Glu Glu Ile Lys Lys Arg Lys Ser Ser Pro Arg Leu
 1090 1095 1100
 Leu Phe Leu Trp Ser Asn Asp Lys Asp Lys Met Arg Ala Arg Ser Leu
 1105 1110 1115 1120
 Ser Thr Ser Leu Ser Leu Val Pro Ala Lys Leu Asp Ser Met Pro Leu
 1125 1130 1135
 Ser Ala Cys Ala Val Tyr Pro Glu Ser Ile Leu Pro Arg Asp Lys His
 1140 1145 1150
 Gly Asp Ile
 1155

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1461 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS

(B) LOCATION: 1..1458

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

| | |
|-----------------------------------------------------------------|-----|
| ATG TCA TTA TTT GAT CAT AAA ATC ACA GAA TTA AAA CAG CTC ATA CAT | 48 |
| Met Ser Leu Phe Asp His Lys Ile Thr Glu Leu Lys Gln Leu Ile His | |
| 1 5 10 15 | |
| AAA AAA GAG ATT AAG ATT TCT GAT CTG GTT GAT GAA TCT TAT AAA CGC | 96 |
| Lys Lys Glu Ile Lys Ile Ser Asp Leu Val Asp Glu Ser Tyr Lys Arg | |
| 20 25 30 | |
| ATC CAA GCG GTT GAT GAT AAG GTA CAA GCC TTT TTG GCA TTA GAT GAA | 144 |
| Ile Gln Ala Val Asp Asp Lys Val Gln Ala Phe Leu Ala Leu Asp Glu | |
| 35 40 45 | |
| GAA AGA GCG CGC GCA TAC GCG AAG GAG CTT GAT GAG GCG GTT GAC GGC | 192 |
| Glu Arg Ala Arg Ala Tyr Ala Lys Glu Leu Asp Glu Ala Val Asp Gly | |
| 50 55 60 | |
| CGT TCT GAG CAC GGT CTT CTT TTC GGT ATG CCG ATC GGC GTA AAA GAT | 240 |
| Arg Ser Glu His Gly Leu Phe Gly Met Pro Ile Gly Val Lys Asp | |
| 65 70 75 80 | |
| AAT ATC GTA ACA AAA GGG CTG CGC ACA ACA TGC TCC AGC AAA ATT CTC | 288 |
| Asn Ile Val Thr Lys Gly Leu Arg Thr Thr Cys Ser Ser Lys Ile Leu | |
| 85 90 95 | |
| GAA AAC TTT GAT CCG ATT TAC GAT GCT ACT GTC GTT CAG CGC CTT CAA | 336 |
| Glu Asn Phe Asp Pro Ile Tyr Asp Ala Thr Val Val Gln Arg Leu Gln | |
| 100 105 110 | |
| GAC GCT GAA GCG GTC ACA ATC GGA AAA CTG AAC ATG GAC GAA TTC GCC | 384 |
| Asp Ala Glu Ala Val Thr Ile Gly Lys Leu Asn Met Asp Glu Phe Ala | |
| 115 120 125 | |
| ATG GGG TCA TCT ACA GAA AAC TCA GCT TAC AAG CTG ACG AAA AAC CCT | 432 |
| Met Gly Ser Ser Thr Glu Asn Ser Ala Tyr Lys Leu Thr Lys Asn Pro | |
| 130 135 140 | |
| TGG AAC CTG GAT ACA GTT CCC GGC GGT TCA AGC GGC GGA TCT GCA GCT | 480 |
| Trp Asn Leu Asp Thr Val Pro Gly Gly Ser Ser Gly Gly Ser Ala Ala | |
| 145 150 155 160 | |
| GCG GTT GCT GCG GGA GAA GTT CCG TTT TCT CTT GGA TCT GAC ACA GGC | 528 |
| Ala Val Ala Ala Gly Glu Val Pro Phe Ser Leu Gly Ser Asp Thr Gly | |
| 165 170 175 | |
| GGC TCC ATC CGT CAG CCG GCA TCT TTC TGC GGC GTT GTC GGA TTA AAA | 576 |
| Gly Ser Ile Arg Gln Pro Ala Ser Phe Cys Gly Val Val Gly Leu Lys | |
| 180 185 190 | |
| CCT ACA TAC GGA CGT GTA TCT CGT TAC GGC CTG GTC GCA TTT GCG TCT | 624 |
| Pro Thr Tyr Gly Arg Val Ser Arg Tyr Gly Leu Val Ala Phe Ala Ser | |
| 195 200 205 | |
| TCA TTG GAC CAA ATC GGA CCG ATT ACA CGT ACG GTT GAG GAT AAC GCG | 672 |
| Ser Leu Asp Gln Ile Gly Pro Ile Thr Arg Thr Val Glu Asp Asn Ala | |
| 210 215 220 | |

| | |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| TTT TTA CTT CAA GCG ATT TCC GGC GTA GAC AAA ATG GAC TCT ACG AGT Phe Leu Leu Gln Ala Ile Ser Gly Val Asp Lys Met Asp Ser Thr Ser 225 230 235 240 | 720 |
| GCA AAT GTG GAC GTG CCT GAT TTT CTT TCT TCA TTA ACT GGC GAC ATC Ala Asn Val Asp Val Pro Asp Phe Leu Ser Ser Leu Thr Gly Asp Ile 245 250 255 | 768 |
| AAA GGA CTG AAA ATC GCC GTT CCG AAA GAA TAC CTT GGT GAA GGT GTC Lys Gly Leu Lys Ile Ala Val Pro Lys Glu Tyr Leu Gly Glu Gly Val 260 265 270 | 816 |
| GGC AAA GAA GCG AGA GAA TCT GTC TTG GCA GCG CTG AAA GTC CTT GAA Gly Lys Glu Ala Arg Glu Ser Val Leu Ala Ala Leu Lys Val Leu Glu 275 280 285 | 864 |
| GGT CTC GGC GCT ACA TGG GAA GAA GTG TCT CTT CCG CAC AGT AAA TAC Gly Leu Gly Ala Thr Trp Glu Glu Val Ser Leu Pro His Ser Lys Tyr 290 295 300 | 912 |
| GCG CTT GCG ACA TAT TAC CTG CTG TCA TCT TCT GAA GCG TCA GCG AAC Ala Leu Ala Thr Tyr Tyr Leu Leu Ser Ser Ser Glu Ala Ser Ala Asn 305 310 315 320 | 960 |
| CTT GCA CGC TTT GAC GGC ATC CGC TAC GGC TAC CGC ACA GAC AAC GCG Leu Ala Arg Phe Asp Gly Ile Arg Tyr Gly Tyr Arg Thr Asp Asn Ala 325 330 335 | 1008 |
| GAT AAC CTG ATC GAC CTT TAC AAG CAA ACG CGC GCT GAA GGT TTC GGA Asp Asn Leu Ile Asp Leu Tyr Lys Gln Thr Arg Ala Glu Gly Phe Gly 340 345 350 | 1056 |
| AAT GAA GTC AAA CGC CGC ATC ATG CTC GGA ACG TTT GCT TTA AGC TCA Asn Glu Val Lys Arg Arg Ile Met Leu Gly Thr Phe Ala Leu Ser Ser 355 360 365 | 1104 |
| GGC TAC TAC GAT GCG TAC TAC AAA AAA GCG CAA AAA GTG CGT ACG TTG Gly Tyr Tyr Asp Ala Tyr Tyr Lys Lys Ala Gln Lys Val Arg Thr Leu 370 375 380 | 1152 |
| ATT AAG AAG GAT TTC GAG GAC GTA TTT GAA AAA TAT GAT GTT ATT GTT Ile Lys Lys Asp Phe Glu Asp Val Phe Glu Lys Tyr Asp Val Ile Val 385 390 395 400 | 1200 |
| GGA CCG ACT ACA CCG ACA CCT GCG TTT AAA ATC GGT GAA AAC ACG AAG Gly Pro Thr Thr Pro Thr Pro Ala Phe Lys Ile Gly Glu Asn Thr Lys 405 410 415 | 1248 |
| GAT CCG CTC ACA ATG TAC GCA AAC GAT ATC TTA ACG ATT CCG GTC AAC Asp Pro Leu Thr Met Tyr Ala Asn Asp Ile Leu Thr Ile Pro Val Asn 420 425 430 | 1296 |
| CTT GCG GCG TAC CGG GAA TCA GGT GCC ATG CGG TTA GCA GAC GGA CTT Leu Ala Ala Tyr Arg Glu Ser Gly Ala Met Arg Leu Ala Asp Gly Leu 435 440 445 | 1344 |
| CCG CTC GGC CTG CAA ATC ATC GGA AAA CAC TTT GAT GAA GCA CTG TAT Pro Leu Gly Leu Gln Ile Ile Gly Lys His Phe Asp Glu Ala Leu Tyr 450 455 460 | 1392 |

ACC GCG TTG CTC ATG CAT TTG AAC AAG CAA CAG ACC ATC ATA AAG CAA 1440
 Thr Ala Leu Leu Met His Leu Asn Lys Gln Gln Thr Ile Ile Lys Gln
 465 470 475 480

AAC CTG AAC TGT AAG GGG TGA 1461
 Asn Leu Asn Cys Lys Gly
 485

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 486 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ser Leu Phe Asp His Lys Ile Thr Glu Leu Lys Gln Leu Ile His
 1 5 10 15
 Lys Lys Glu Ile Lys Ile Ser Asp Leu Val Asp Glu Ser Tyr Lys Arg
 20 25 30
 Ile Gln Ala Val Asp Asp Lys Val Gln Ala Phe Leu Ala Leu Asp Glu
 35 40 45
 Glu Arg Ala Arg Ala Tyr Ala Lys Glu Leu Asp Glu Ala Val Asp Gly
 50 55 60
 Arg Ser Glu His Gly Leu Phe Gly Met Pro Ile Gly Val Lys Asp
 65 70 75 80
 Asn Ile Val Thr Lys Gly Leu Arg Thr Thr Cys Ser Ser Lys Ile Leu
 85 90 95
 Glu Asn Phe Asp Pro Ile Tyr Asp Ala Thr Val Val Gln Arg Leu Gln
 100 105 110
 Asp Ala Glu Ala Val Thr Ile Gly Lys Leu Asn Met Asp Glu Phe Ala
 115 120 125
 Met Gly Ser Ser Thr Glu Asn Ser Ala Tyr Lys Leu Thr Lys Asn Pro
 130 135 140
 Trp Asn Leu Asp Thr Val Pro Gly Gly Ser Ser Gly Gly Ser Ala Ala
 145 150 155 160
 Ala Val Ala Ala Gly Glu Val Pro Phe Ser Leu Gly Ser Asp Thr Gly
 165 170 175
 Gly Ser Ile Arg Gln Pro Ala Ser Phe Cys Gly Val Val Gly Leu Lys
 180 185 190
 Pro Thr Tyr Gly Arg Val Ser Arg Tyr Gly Leu Val Ala Phe Ala Ser
 195 200 205
 Ser Leu Asp Gln Ile Gly Pro Ile Thr Arg Thr Val Glu Asp Asn Ala
 210 215 220

Phe Leu Leu Gln Ala Ile Ser Gly Val Asp Lys Met Asp Ser Thr Ser
 225 230 235 240
 Ala Asn Val Asp Val Pro Asp Phe Leu Ser Ser Leu Thr Gly Asp Ile
 245 250 255
 Lys Gly Leu Lys Ile Ala Val Pro Lys Glu Tyr Leu Gly Glu Gly Val
 260 265 270
 Gly Lys Glu Ala Arg Glu Ser Val Leu Ala Ala Leu Lys Val Leu Glu
 275 280 285
 Gly Leu Gly Ala Thr Trp Glu Glu Val Ser Leu Pro His Ser Lys Tyr
 290 295 300
 Ala Leu Ala Thr Tyr Tyr Leu Leu Ser Ser Ser Glu Ala Ser Ala Asn
 305 310 315 320
 Leu Ala Arg Phe Asp Gly Ile Arg Tyr Gly Tyr Arg Thr Asp Asn Ala
 325 330 335
 Asp Asn Leu Ile Asp Leu Tyr Lys Gln Thr Arg Ala Glu Gly Phe Gly
 340 345 350
 Asn Glu Val Lys Arg Arg Ile Met Leu Gly Thr Phe Ala Leu Ser Ser
 355 360 365
 Gly Tyr Tyr Asp Ala Tyr Tyr Lys Lys Ala Gln Lys Val Arg Thr Leu
 370 375 380
 Ile Lys Lys Asp Phe Glu Asp Val Phe Glu Lys Tyr Asp Val Ile Val
 385 390 395 400
 Gly Pro Thr Thr Pro Thr Pro Ala Phe Lys Ile Gly Glu Asn Thr Lys
 405 410 415
 Asp Pro Leu Thr Met Tyr Ala Asn Asp Ile Leu Thr Ile Pro Val Asn
 420 425 430
 Leu Ala Ala Tyr Arg Glu Ser Gly Ala Met Arg Leu Ala Asp Gly Leu
 435 440 445
 Pro Leu Gly Leu Gln Ile Ile Gly Lys His Phe Asp Glu Ala Leu Tyr
 450 455 460
 Thr Ala Leu Leu Met His Leu Asn Lys Gln Gln Thr Ile Ile Lys Gln
 465 470 475 480
 Asn Leu Asn Cys Lys Gly
 485

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1431 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

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(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..1428

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

| | |
|-----------------------------------------------------------------|-----|
| TTG AAC TTT GAA ACG GTA ATC GGA CTT GAA GTC CAC GTT GAG TTA AAA | 48 |
| Leu Asn Phe Glu Thr Val Ile Gly Leu Glu Val His Val Glu Leu Lys | |
| 1 5 10 15 | |
| ACA AAA TCA AAA ATT TTC TCA AGC TCT CCA ACG CCA TTC GGC GCG GAG | 96 |
| Thr Lys Ser Lys Ile Phe Ser Ser Ser Pro Thr Pro Phe Gly Ala Glu | |
| 20 25 30 | |
| GCG AAT ACG CAG ACA AGC GTT ATT GAC CTC GGA TAT CCG GGC GTC CTG | 144 |
| Ala Asn Thr Gln Thr Ser Val Ile Asp Leu Gly Tyr Pro Gly Val Leu | |
| 35 40 45 | |
| CCT GTT CTG AAC AAA GAA GCC GTT GAA TTC GCA ATG AAA GCC GCT ATG | 192 |
| Pro Val Leu Asn Lys Glu Ala Val Glu Phe Ala Met Lys Ala Ala Met | |
| 50 55 60 | |
| GCG CTC AAC TGT GAG ATC GCA ACG GAT ACG AAG TTT GAC CGC AAA AAC | 240 |
| Ala Leu Asn Cys Glu Ile Ala Thr Asp Thr Lys Phe Asp Arg Lys Asn | |
| 65 70 75 80 | |
| TAT TTC TAT CCT GAC AAC CCG AAA GCG TAT CAG ATT TCT CAA TTT GAT | 288 |
| Tyr Phe Tyr Pro Asp Asn Pro Lys Ala Tyr Gln Ile Ser Gln Phe Asp | |
| 85 90 95 | |
| AAG CCA ATC GGC GAA AAC GGC TGG ATC GAA ATT GAA GTC GGC GGC AAA | 336 |
| Lys Pro Ile Gly Glu Asn Gly Trp Ile Glu Ile Glu Val Gly Gly Lys | |
| 100 105 110 | |
| ACA AAA CGC ATC GGC ATC ACG CGC CTT CAT CTT GAA GAG GAT GCC GGA | 384 |
| Thr Lys Arg Ile Gly Ile Thr Arg Leu His Leu Glu Glu Asp Ala Gly | |
| 115 120 125 | |
| AAA CTG ACG CAT ACG GGC GAC GGC TAT TCT CTT GTT GAC TTC AAC CGT | 432 |
| Lys Leu Thr His Thr Gly Asp Gly Tyr Ser Leu Val Asp Phe Asn Arg | |
| 130 135 140 | |
| CAA GGA ACG CCG CTT GTT GAG TNC GTA TCA GAG CCG GAC ATC CGC ACG | 480 |
| Gln Gly Thr Pro Leu Val Glu Xaa Val Ser Glu Pro Asp Ile Arg Thr | |
| 145 150 155 160 | |
| CCG GAA GAA NCG TAC GCA TAT CTT GAA AAG CTG AAA TCC ATC ATC CAA | 528 |
| Pro Glu Glu Xaa Tyr Ala Tyr Leu Glu Lys Leu Lys Ser Ile Ile Gln | |
| 165 170 175 | |
| TAT ACA GGC GTT TCT GAC TGT AAA ATG GAA GAA GGC TCA CTT CGC TGT | 576 |
| Tyr Thr Gly Val Ser Asp Cys Lys Met Glu Glu Gly Ser Leu Arg Cys | |
| 180 185 190 | |
| GAC GCC AAT ATC TCT CTT CGT CCG ATC GGC CAA GAG GAA TTC GGC ACA | 624 |
| Asp Ala Asn Ile Ser Leu Arg Pro Ile Gly Gln Glu Glu Phe Gly Thr | |
| 195 200 205 | |

| | |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| AAA ACA GAA TTG AAA AAC TTG AAC TCC TTT GCG TTT GTT CAA AAA GGC Lys Thr Glu Leu Lys Asn Leu Asn Ser Phe Ala Phe Val Gln Lys Gly 210 215 220 | 672 |
| CTT GAG CAT GAA GAA AAA CGC CAG GAG CAG GTT CTT CTT TCC GGC TTC Leu Glu His Glu Glu Lys Arg Gln Glu Gln Val Leu Leu Ser Gly Phe 225 230 235 240 | 720 |
| TTC ATC CAG CAA GAA ACT CGC CGT TAT GAT GAA GCA ACG AAG AAA ACC Phe Ile Gln Gln Glu Thr Arg Arg Tyr Asp Glu Ala Thr Lys Lys Thr 245 250 255 | 768 |
| ATT CTT ATG CGT GTC AAA GAG GGA TCT GAC GAC TAC CGT TAC TTC CCA Ile Leu Met Arg Val Lys Glu Gly Ser Asp Asp Tyr Arg Tyr Phe Pro 260 265 270 | 816 |
| GAG CCA GAT CTA GTC GAG CTC TAC ATT GAT GAT GAA TGG AAG GAA CGC Glu Pro Asp Leu Val Glu Leu Tyr Ile Asp Asp Glu Trp Lys Glu Arg 275 280 285 | 864 |
| GTA AAA GCA AGC ATT CCT GAG CTT CCG GAT GAG CGC CGC AAG CGT TAT Val Lys Ala Ser Ile Pro Glu Leu Pro Asp Glu Arg Arg Lys Arg Tyr 290 295 300 | 912 |
| ATC GAA GAG CTT GGC TTC GCT GCA TAT GAC GCA ATG GTT CTG ACG CTG Ile Glu Glu Leu Gly Phe Ala Ala Tyr Asp Ala Met Val Leu Thr Leu 305 310 315 320 | 960 |
| ACA AAA GAA ATG GCT GAT TTC TTC GAA GAA ACC GTT CAA AAA GGC GCT Thr Lys Glu Met Ala Asp Phe Phe Glu Glu Thr Val Gln Lys Gly Ala 325 330 335 | 1008 |
| GAA GCT AAA CAA GCG TCT AAC TGG CTG ATG GGT GAA GTG TCA GCT TAC Glu Ala Lys Gln Ala Ser Asn Trp Leu Met Gly Glu Val Ser Ala Tyr 340 345 350 | 1056 |
| CTA AAC GCA GAA CAA AAA GAG CTT GCC GAT GTT GCC CTG ACA CCT GAA Leu Asn Ala Glu Gln Lys Glu Leu Ala Asp Val Ala Leu Thr Pro Glu 355 360 365 | 1104 |
| GGC CTT GCC GGC ATG ATC AAA TTG ATT GAA AAA GGA ACC ATT TCT TCT Gly Leu Ala Gly Met Ile Lys Leu Ile Glu Lys Gly Thr Ile Ser Ser 370 375 380 | 1152 |
| AAG ATC GCG AAG AAA GTG TTT AAA GAA TTG ATT GAA AAA GGC GGC GAC Lys Ile Ala Lys Lys Val Phe Lys Glu Leu Ile Glu Lys Gly Thr Ile Ser Ser 385 390 395 400 | 1200 |
| GCT GAG AAG ATT GTG AAA GAG AAA GGC CTT GTT CAG ATT TCT GAC GAA Ala Glu Lys Ile Val Lys Glu Lys Gly Leu Val Gln Ile Ser Asp Glu 405 410 415 | 1248 |
| GGC GTG CTT CTG AAG CTT GTC ACT GAG GCG CTT GAC AAC AAT CCT CAA Gly Val Leu Leu Lys Leu Val Thr Glu Ala Leu Asp Asn Asn Pro Gln 420 425 430 | 1296 |
| TCA ATC GAA GAC TTT AAA AAC GGA AAA GAC CGC GCG ATC GGC TTC CTA Ser Ile Glu Asp Phe Lys Asn Gly Lys Asp Arg Ala Ile Gly Phe Leu 435 440 445 | 1344 |

GTC GGA CAG ATT ATG AAA GCG TCC AAA GGA CAA GCC AAC CCG CCG ATG 1392
 Val Gly Gln Ile Met Lys Ala Ser Lys Gly Gln Ala Asn Pro Pro Met
 450 455 460

GTC AAC AAA ATT CTG CTT GAA GAA ATT AAA AAA CGC TAA 1431
 Val Asn Lys Ile Leu Leu Glu Glu Ile Lys Lys Arg
 465 470 475

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 476 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu Asn Phe Glu Thr Val Ile Gly Leu Glu Val His Val Glu Leu Lys
 1 5 10 15
 Thr Lys Ser Lys Ile Phe Ser Ser Ser Pro Thr Pro Phe Gly Ala Glu
 20 25 30
 Ala Asn Thr Gln Thr Ser Val Ile Asp Leu Gly Tyr Pro Gly Val Leu
 35 40 45
 Pro Val Leu Asn Lys Glu Ala Val Glu Phe Ala Met Lys Ala Ala Met
 50 55 60
 Ala Leu Asn Cys Glu Ile Ala Thr Asp Thr Lys Phe Asp Arg Lys Asn
 65 70 75 80
 Tyr Phe Tyr Pro Asp Asn Pro Lys Ala Tyr Gln Ile Ser Gln Phe Asp
 85 90 95
 Lys Pro Ile Gly Glu Asn Gly Trp Ile Glu Ile Glu Val Gly Gly Lys
 100 105 110
 Thr Lys Arg Ile Gly Ile Thr Arg Leu His Leu Glu Glu Asp Ala Gly
 115 120 125
 Lys Leu Thr His Thr Gly Asp Gly Tyr Ser Leu Val Asp Phe Asn Arg
 130 135 140
 Gln Gly Thr Pro Leu Val Glu Xaa Val Ser Glu Pro Asp Ile Arg Thr
 145 150 155 160
 Pro Glu Glu Xaa Tyr Ala Tyr Leu Glu Lys Leu Lys Ser Ile Ile Gln
 165 170 175
 Tyr Thr Gly Val Ser Asp Cys Lys Met Glu Glu Gly Ser Leu Arg Cys
 180 185 190
 Asp Ala Asn Ile Ser Leu Arg Pro Ile Gly Gln Glu Glu Phe Gly Thr
 195 200 205
 Lys Thr Glu Leu Lys Asn Leu Asn Ser Phe Ala Phe Val Gln Lys Gly
 210 215 220

Leu Glu His Glu Glu Lys Arg Gln Glu Gln Val Leu Leu Ser Gly Phe
 225 230 235 240
 Phe Ile Gln Gln Glu Thr Arg Arg Tyr Asp Glu Ala Thr Lys Lys Thr
 245 250 255
 Ile Leu Met Arg Val Lys Glu Gly Ser Asp Asp Tyr Arg Tyr Phe Pro
 260 265 270
 Glu Pro Asp Leu Val Glu Leu Tyr Ile Asp Asp Glu Trp Lys Glu Arg
 275 280 285
 Val Lys Ala Ser Ile Pro Glu Leu Pro Asp Glu Arg Arg Lys Arg Tyr
 290 295 300
 Ile Glu Glu Leu Gly Phe Ala Ala Tyr Asp Ala Met Val Leu Thr Leu
 305 310 315 320
 Thr Lys Glu Met Ala Asp Phe Phe Glu Glu Thr Val Gln Lys Gly Ala
 325 330 335
 Glu Ala Lys Gln Ala Ser Asn Trp Leu Met Gly Glu Val Ser Ala Tyr
 340 345 350
 Leu Asn Ala Glu Gln Lys Glu Leu Ala Asp Val Ala Leu Thr Pro Glu
 355 360 365
 Gly Leu Ala Gly Met Ile Lys Leu Ile Glu Lys Gly Thr Ile Ser Ser
 370 375 380
 Lys Ile Ala Lys Lys Val Phe Lys Glu Leu Ile Glu Lys Gly Gly Asp
 385 390 395 400
 Ala Glu Lys Ile Val Lys Glu Lys Gly Leu Val Gln Ile Ser Asp Glu
 405 410 415
 Gly Val Leu Leu Lys Leu Val Thr Glu Ala Leu Asp Asn Asn Pro Gln
 420 425 430
 Ser Ile Glu Asp Phe Lys Asn Gly Lys Asp Arg Ala Ile Gly Phe Leu
 435 440 445
 Val Gly Gln Ile Met Lys Ala Ser Lys Gly Gln Ala Asn Pro Pro Met
 450 455 460
 Val Asn Lys Ile Leu Leu Glu Glu Ile Lys Lys Arg
 465 470 475

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 291 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 1..288

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

| | |
|-----------------------------------------------------------------|-----|
| ATG TCA CGA ATT TCA ATA GAA GAA GTA AAG CAC GTT GCG CAC CTT GCA | 48 |
| Met Ser Arg Ile Ser Ile Glu Glu Val Lys His Val Ala His Leu Ala | |
| 1 5 10 15 | |
| AGA CTT GCG ATT ACT GAA GAA GAA GCA AAA ATG TTC ACT GAA CAG CTC | 96 |
| Arg Leu Ala Ile Thr Glu Glu Glu Ala Lys Met Phe Thr Glu Gln Leu | |
| 20 25 30 | |
| GAC AGT ATC ATT TCA TTT GCC GAG GAG CTT AAT GAG GTT AAC ACA GAC | 144 |
| Asp Ser Ile Ile Ser Phe Ala Glu Glu Leu Asn Glu Val Asn Thr Asp | |
| 35 40 45 | |
| AAT GTG GAG CCT ACA ACT CAC GTG CTG AAA ATG AAA AAT GTC ATG AGA | 192 |
| Asn Val Glu Pro Thr Thr His Val Leu Lys Met Lys Asn Val Met Arg | |
| 50 55 60 | |
| GAA GAT GAA GCG GGT AAA GGT CTT CCG GTT GAG GAT GTC ATG AAA AAT | 240 |
| Glu Asp Glu Ala Gly Lys Gly Leu Pro Val Glu Asp Val Met Lys Asn | |
| 65 70 75 80 | |
| GCG CCT GAC CAT AAA GAC GGC TAT ATT CGT GTG CCA TCA ATT CTG GAC | 288 |
| Ala Pro Asp His Lys Asp Gly Tyr Ile Arg Val Pro Ser Ile Leu Asp | |
| 85 90 95 | |
| TAA | 291 |

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 96 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

| | |
|-----------------------------------------------------------------|--|
| Met Ser Arg Ile Ser Ile Glu Glu Val Lys His Val Ala His Leu Ala | |
| 1 5 10 15 | |
| Arg Leu Ala Ile Thr Glu Glu Glu Ala Lys Met Phe Thr Glu Gln Leu | |
| 20 25 30 | |
| Asp Ser Ile Ile Ser Phe Ala Glu Glu Leu Asn Glu Val Asn Thr Asp | |
| 35 40 45 | |
| Asn Val Glu Pro Thr Thr His Val Leu Lys Met Lys Asn Val Met Arg | |
| 50 55 60 | |
| Glu Asp Glu Ala Gly Lys Gly Leu Pro Val Glu Asp Val Met Lys Asn | |
| 65 70 75 80 | |
| Ala Pro Asp His Lys Asp Gly Tyr Ile Arg Val Pro Ser Ile Leu Asp | |
| 85 90 95 | |

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Claims

1. An isolated polynucleotide encoding an amidotransferase (AdT) protein.
2. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:
 - (a) a polynucleotide having at least a 70% identity to a polynucleotide encoding a polypeptide encoded by SEQ ID NO:1;
 - (b) a polynucleotide which is complementary to the polynucleotide of (a);
and,
 - (c) a polynucleotide comprising at least 15 sequential bases of the polynucleotide of (a) or (b).
3. The polynucleotide of claim 2 wherein the polynucleotide is DNA.
4. The polynucleotide of claim 2 wherein the polynucleotide is RNA.
5. The polynucleotide of claim 3 comprising the nucleotides 103 to 3306 set forth in SEQ ID NO:1.
6. A vector comprising the DNA of claim 3.
7. A host cell comprising the vector of claim 6.

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8. A process for producing a polypeptide comprising expressing from the host cell of claim 7 a polypeptide encoded by said DNA.
9. A process for producing a cell which expresses a polypeptide comprising transforming or transfecting the cell with the vector of claim 6 such that the cell expresses the polypeptide encoded by the DNA contained in the vector.
10. A process for producing an amidotransferase polypeptide or amidotransferase fragment comprising culturing a host cell of claim 7 under conditions sufficient for the production of said polypeptide or fragment.
11. A polypeptide comprising an amino acid sequence which is at least 70% identical to a polypeptide encoded by nucleotides 103 to 3306 set forth in SEQ ID NO:1.
12. An antibody, or antibody fragment containing an antigen binding site, wherein said antibody binds to a polypeptide of claim 11.
13. An antagonist which inhibits the activity of the polypeptide of claim 11.

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14. A method for the treatment of an individual having need of amidotransferase comprising administering to the individual a therapeutically effective amount of the polypeptide of claim 11.
15. The method of claim 14 wherein said therapeutically effective amount of the polypeptide is administered by providing to the individual DNA encoding said polypeptide and expressing said polypeptide *in vivo*.
16. A method for the treatment of an individual having need to inhibit amidotransferase polypeptide comprising administering to the individual a therapeutically effective amount of the antagonist of claim 13.
17. A process for diagnosing a disease related to expression of the polypeptide of claim 11 comprising determining a nucleic acid sequence encoding said polypeptide.
18. A diagnostic process comprising analyzing for the presence of the polypeptide of claim 11 in a sample derived from a host.
19. A method for identifying compounds which bind to and inhibit an activity of a polypeptide of claim 11 comprising:
 - (a) incubating a first sample of the polypeptide and its substrate;

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- (b) measuring an uninhibited reactivity of the polypeptide from step (a);
- (c) incubating a first sample of the polypeptide and its substrate in the presence of a second sample comprising an inhibitor compound;
- (d) measuring an inhibited reactivity of the polypeptide from step (c); and,
- (e) comparing the inhibited reactivity to the uninhibited reactivity of the polypeptide.

20. A method for identifying compounds which bind to and inhibit an activity of a polypeptide of claim 11 comprising:

- (a) contacting a cell expressing on the surface thereof a binding site for the polypeptide, said binding being associated with a second component capable of providing a detectable signal in response to the binding of a compound to said binding site, with a compound to be screened under conditions to permit binding to the binding site; and
- (b) determining whether the compound binds to and activates or inhibits the binding by detecting the presence or absence of a signal generated from the interaction of the compound with the binding site.

21. A method for identifying inhibitor-resistant AdT mutants comprising:

- (a) incubating a first sample of wild-type AdT and its substrate in the presence of a second sample comprising an AdT inhibitor;
- (b) measuring an unmutated reactivity of the AdT from step (a);

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- (c) incubating a first sample of a mutated AdT and its substrate in the presence of a second sample comprising an AdT inhibitor;
 - (d) measuring a mutated reactivity of the mutated AdT from step (c); and,
 - (e) comparing the mutated reactivity to the unmutated reactivity of the wild-type AdT.
22. A method for inducing an immunological response in a mammal which comprises inoculating the mammal with amidotransferase, or a fragment or variant thereof, adequate to produce antibody to protect said animal from disease.
23. A method of inducing immunological response in a mammal which comprises, through gene therapy, delivering a gene encoding an amidotransferase fragment or a variant thereof, for expressing amidotransferase, or a fragment or a variant thereof *in vivo* in order to induce an immunological response to produce antibody to protect said animal from disease.
24. An immunological composition comprising a DNA which codes for and expresses an amidotransferase polynucleotide or protein coded therefrom which, when introduced into a mammal, induces an immunological response in the mammal to a given amidotransferase polynucleotide or protein coded therefrom.

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25. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:
- (a) a polynucleotide having at least a 70% identity to a polynucleotide encoding a polypeptide comprising SEQ ID NO:4;
 - (b) a polynucleotide which is complementary to the polynucleotide of (a);
and,
 - (c) a polynucleotide comprising at least 15 sequential bases of the polynucleotide of (a) or (b).
26. A vector comprising the polynucleotide of claim 25.
27. A host cell comprising the vector of claim 26.
28. A process for producing a polypeptide comprising expressing from the host cell of claim 27 a polypeptide encoded by said polynucleotide.
29. A polypeptide comprising an amino acid sequence which is at least 70% identical to the amino acid set forth in SEQ ID NO:4.
30. An antibody, or antibody fragment containing an antigen binding site, wherein said antibody binds to a polypeptide of claim 29.

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31. An antagonist which inhibits the activity of the polypeptide of claim 29.
32. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:
 - (a) a polynucleotide having at least a 70% identity to a polynucleotide encoding a polypeptide comprising SEQ ID NO:6;
 - (b) a polynucleotide which is complementary to the polynucleotide of (a);
and,
 - (c) a polynucleotide comprising at least 15 sequential bases of the polynucleotide of (a) or (b).
33. A vector comprising the polynucleotide of claim 32.
34. A host cell comprising the vector of claim 33.
35. A process for producing a polypeptide comprising expressing from the host cell of claim 34 a polypeptide encoded by said polynucleotide..
36. A polypeptide comprising an amino acid sequence which is at least 70% identical to the amino acid set forth in SEQ ID NO:6.

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37. An antibody, or antibody fragment containing an antigen binding site, wherein said antibody binds to a polypeptide of claim 36.
38. An antagonist which inhibits the activity of the polypeptide of claim 36.
39. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:
- (a) a polynucleotide having at least a 70% identity to a polynucleotide encoding a polypeptide comprising SEQ ID NO:8;
 - (b) a polynucleotide which is complementary to the polynucleotide of (a); and,
 - (c) a polynucleotide comprising at least 15 sequential bases of the polynucleotide of (a) or (b).
40. A vector comprising the polynucleotide of claim 39.
41. A host cell comprising the vector of claim 40.
42. A process for producing a polypeptide comprising expressing from the host cell of claim 41 a polypeptide encoded by said polynucleotide.

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43. A polypeptide comprising an amino acid sequence which is at least 70% identical to the amino acid set forth in SEQ ID NO:8.
44. An antibody, or antibody fragment containing an antigen binding site, wherein said antibody binds to a polypeptide of claim 43.
45. An antagonist which inhibits the activity of the polypeptide of claim 43.
46. An isolated heterotrimeric protein comprising subunits A, B, and C, wherein:
 - said subunit A has an amino acid sequence selected from the group consisting of SEQ ID NO:4, an allelic variant of SEQ ID NO:4, a conservative substitution variant of SEQ ID NO:4, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:4 and encodes a subunit of a heterotrimeric amidotransferase;
 - said subunit B has an amino acid sequence selected from the group consisting of SEQ ID NO:6, an allelic variant of SEQ ID NO:6, a conservative substitution variant of SEQ ID NO:6, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:6 and encodes a subunit of a heterotrimeric amidotransferase;
 - said subunit C has an amino acid sequence selected from the group consisting of SEQ ID NO:8, an allelic variant of SEQ ID NO:8, a conservative substitution variant of SEQ ID NO:8, and an amino acid sequence that is encoded by a nucleic

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acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:8 and encodes a subunit of a heterotrimeric amidotransferase.

47. An antibody, or antibody fragment containing an antigen binding site, wherein said antibody binds to a protein of claim 46.

48. An isolated nucleic acid molecule that encodes a protein of claim 46.

49. A recombinant host that has been altered to contain a nucleic acid molecule of claim 48.

50. A method for producing an AdT protein comprising the step of culturing the host of claim 49 under conditions in which said introduced nucleic acid molecule is expressed.

51. A method to identify an agent that blocks translation, said method comprising the steps of:

- (a) contacting an agent with an AdT protein, or a subunit thereof; and,
- (b) determining whether said agent binds to said AdT protein or said

subunit;

wherein said translation blocking agent is identified as being able to bind to said AdT protein, or said subunit.

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52. The method of claim 51, wherein said AdT protein comprises a heterotrimeric protein consisting of an A, B and C subunit, wherein:

said subunit A has an amino acid sequence selected from the group consisting of SEQ ID NO:4, an allelic variant of SEQ ID NO:4, a conservative substitution variant of SEQ ID NO:4, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:4 and encodes a subunit of a heterotrimeric amidotransferase;

said subunit B has an amino acid sequence selected from the group consisting of SEQ ID NO:6, an allelic variant of SEQ ID NO:6, a conservative substitution variant of SEQ ID NO:6, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:6 and encodes a subunit of a heterotrimeric amidotransferase;

said subunit C has an amino acid sequence selected from the group consisting of SEQ ID NO:8, an allelic variant of SEQ ID NO:8, a conservative substitution variant of SEQ ID NO:8, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:8 and encodes a subunit of a heterotrimeric amidotransferase.

53. The method of claim 52, wherein a single subunit of said AdT protein is used, and:

if subunit A is used, said subunit A has an amino acid sequence selected from the group consisting of SEQ ID NO:4, an allelic variant of SEQ ID NO:4, a

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conservative substitution variant of SEQ ID NO:4, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:4 and encodes a subunit of a heterotrimeric amidotransferase;

if subunit B is used, said subunit B has an amino acid sequence selected from the group consisting of SEQ ID NO:6, an allelic variant of SEQ ID NO:6, a conservative substitution variant of SEQ ID NO:6, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:6 and encodes a subunit of a heterotrimeric amidotransferase;

if subunit C is used, said subunit C has an amino acid sequence selected from the group consisting of SEQ ID NO:8, an allelic variant of SEQ ID NO:8, a conservative substitution variant of SEQ ID NO:8, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:8 and encodes a subunit of a heterotrimeric amidotransferase.

54. The method of claim 51, wherein said agent is further tested for the ability to block the activity of said AdT protein.

55. The method of claim 54, wherein said AdT activity is tested in a cell free system.

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56. The method of claim 54, wherein said AdT activity is tested in a cellular system.

57. A method to identify an agent that blocks translation, said method comprising the steps of:

- (a) contacting an agent with one or more of the subunits of an AdT protein;
 - (b) incubating the three subunits of an AdT protein under conditions in which said subunits would associate to form an active AdT protein, wherein at least one of said subunits is from step (a);
 - (c) determining whether said agent blocks the association of said three subunits;
- wherein said translation blocking agent is identified as being able to block the association of the subunits of said AdT protein.

58. The method of claim 57, wherein said AdT protein comprises a heterotrimeric protein consisting of an A, B and C subunit, wherein:

said subunit A has an amino acid sequence selected from the group consisting of SEQ ID NO:4, an allelic variant of SEQ ID NO:4, a conservative substitution variant of SEQ ID NO:4, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:4 and encodes a subunit of a heterotrimeric amidotransferase;

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said subunit B has an amino acid sequence selected from the group consisting of SEQ ID NO:6, an allelic variant of SEQ ID NO:6, a conservative substitution variant of SEQ ID NO:6, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:6 and encodes a subunit of a heterotrimeric amidotransferase;

said subunit C has an amino acid sequence selected from the group consisting of SEQ ID NO:8, an allelic variant of SEQ ID NO:8, a conservative substitution variant of SEQ ID NO:8, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:8 and encodes a subunit of a heterotrimeric amidotransferase.

59. The method of claim 57, wherein said agent is further tested for the ability to block the activity of said AdT protein.

60. The method of claim 59, wherein said AdT protein activity is tested in a cell free system.

61. The method of claim 59, wherein said AdT protein activity is tested in a cellular system.

62. A method to identify an agent that blocks translation, said method comprising the steps of:

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- (a) contacting an agent with an AdT protein;
- (b) determining whether said agent blocks the activity of said AdT protein.

63. The method of claim 62, wherein said AdT protein comprises a heterotrimeric protein consisting of an A, B and C subunit, wherein:

said subunit A has an amino acid sequence selected from the group consisting of SEQ ID NO:4, an allelic variant of SEQ ID NO:4, a conservative substitution variant of SEQ ID NO:4, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:4 and encodes a subunit of a heterotrimeric amidotransferase;

said subunit B has an amino acid sequence selected from the group consisting of SEQ ID NO:6, an allelic variant of SEQ ID NO:6, a conservative substitution variant of SEQ ID NO:6, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:6 and encodes a subunit of a heterotrimeric amidotransferase;

said subunit C has an amino acid sequence selected from the group consisting of SEQ ID NO:8, an allelic variant of SEQ ID NO:8, a conservative substitution variant of SEQ ID NO:8, and an amino acid sequence that is encoded by a nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule encoding SEQ ID NO:8 and encodes a subunit of a heterotrimeric amidotransferase.

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64. The method of claim 63, wherein said AdT protein activity is tested in a cell free system.
65. The method of claim 63, wherein said AdT protein activity is tested in a cellular system.
66. A method to block translation of a protein within a cell, comprising the step of contacting said cell with an amount of an agent that binds to an AdT protein, or a subunit thereof, sufficient to block said translation.
67. The method of claim 66, wherein said agent binds to a subunit of said AdT and blocks the association of said subunits.
68. The method of claim 66, wherein said agent is used as an antibacterial agent.
69. The method of claim 66, wherein said agent is used as an antifungal agent.
70. The method of claim 66, wherein said agent is used as a herbicide.
71. An isolated polynucleotide that codes for a mutant AdT which confers resistance to an inhibitor of wild-type AdT.

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72. A vector comprising the polynucleotide of claim 71.
73. A host cell comprising the vector of claim 72.
74. The host cell of claim 73 wherein the host cell comprises a plant cell.
75. A process for producing a polypeptide comprising expressing from the host cell of claim 73 a polypeptide encoded by said polynucleotide.
76. A process for producing a cell which expresses a polypeptide comprising transforming or transfecting the cell with the vector of claim 73 such that the cell expresses the polypeptide encoded by the polynucleotide contained in the vector.
77. A process for producing a plant which comprises a gene for resistance to an AdT inhibitor, said process comprising regenerating a plant from the plant cell of claim 74.
78. A process of plant husbandry comprising:
- (a) planting a plant which comprises a gene for resistance to an AdT inhibitor;
 - (b) applying a herbicide which comprises an AdT inhibitor.

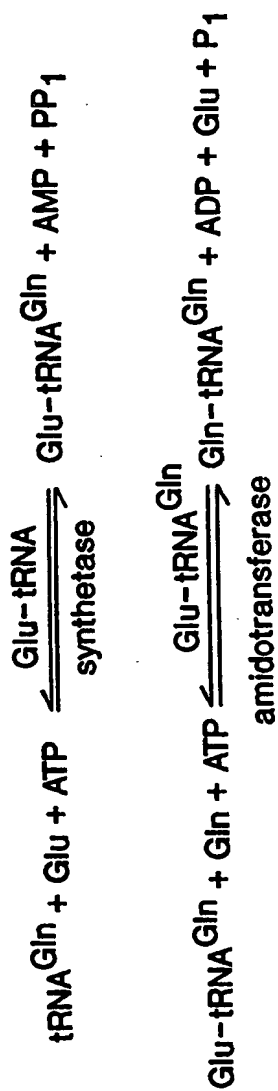


FIG. 1

pABC (pBluescript KS-ABC, 6.4kb, Ap^r)

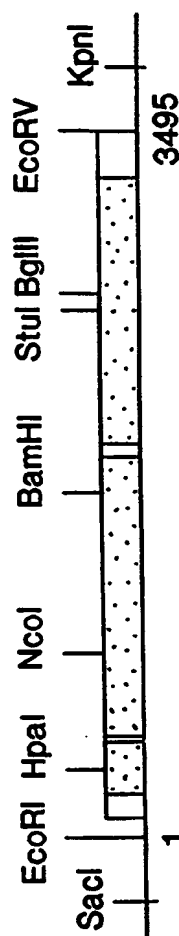


FIG. 2

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| | | | | | |
|------|----------------------------|------------|-------------|-------------|------------|
| 1 | 1 GAATTCGATC (EcoRI) | CTGTCTCAAG | GCGTTTTGTT | GCTTTAAAGG | GCTTGTTTTT |
| 51 | GATATGATCA C START→ | GTATTATATG | ACTTAACGGA | GAAATATGTG | GAGGTGGATC |
| 101 | ATATGTCACG | AATTTCAATA | GAAGAAGTAA | AGCACGTTGC | GCACCTTGCA |
| 151 | AGACTTGCGA | TTACTGAAGA | AGAAGCAAAA | ATGTTCACTG | AACAGCTCGA |
| 201 | CAGTATCATT | TCATTTGCCG | AGGAGCTTAA | TGAGGTTAAC | ACAGACAATG |
| 251 | TGGAGCCTAC | AACTCACGTG | CTGAAAATGA | AAAATGTCAT | GAGAGAAGAT |
| 301 | GAAGCGGGTA | AAGGTCTTCC | GGTTGAGGAT | GTCATGAAAA | ATGCGCCTGA |
| 351 | CCATAAAGAC A START→ | GGCTATATTC | GTGTGCCATC | AATCTGGAC | TAAAGGAGGG |
| 401 | ACACAAGAAT | GTCATTATTT | GATCATAAAA | TCACAGAATT | AAAACAGCTC |
| 451 | ATACATAAAA | AAGAGATTAA | GATTTCTGAT | CTGGTTGATG | AATCTTATAA |
| 501 | ACGCATCCAA | GCGGTTGATG | ATAAGGTACA | AGCCTTTTTG | GCATTAGATG |
| 551 | AAGAAAGACG | CGCGGCATAC | GCGAAGGAGC | TTGATGAGGC | GGTTGACGGC |
| 601 | CGTTCTGAGC | ACGGTCTTCT | TTTCGGTATG | CCGATCGGCG | TAAAAGATAA |
| 651 | TATCGTAACA | AAAGGGCTGC | GCACAACATG | CTCCAGCAAA | ATTCTCGAAA |
| 701 | ACTTTGATCC | GATTTACGAT | GCTACTGTCTG | TTCAGCGCCT | TCAAGACGCT |
| 751 | GAAGCGGTCA | CAATCGGAAA | ACTGAACATG | GACGAATTCTG | CCATGGGGTC |
| 801 | ATCTACAGAA | AACTCAGCTT | ACAAGCTGAC | GAAAAACCCT | TGGAACCTGG |
| 851 | ATACAGTTCC | CGGCGGTTCA | AGCGGCGGAT | CTGCAGCTGC | GGTTGCTGCG |
| 901 | GGAGAAGTTC | CGTTTCTCT | TGGATCTGAC | ACAGGCGGCT | CCATCCGTCA |
| 951 | GCCGGCATCT | TTCTGCGGCG | TTGTCTGATT | AAAACCTACA | TACGGACGTG |
| 1001 | TATCTCGTTA | CGGCCTGGTC | GCATTTGCGT | CTTCATTGGA | CCAAATCGGA |
| 1051 | CCGATTACAC | GTACGGTTGA | GGATAACGCG | TTTTTACTTC | AAGCGATTTC |
| 1101 | CGGCGTAGAC | AAAATGGACT | CTACGAGTGC | AAATGTGGAC | GTGCCTGATT |
| 1151 | TTCTTTCTTC | ATTAAGTGGC | GACATCAAAG | GACTGAAAAT | CGCCGTTCGG |
| 1201 | AAAGAATACC | TTGGTGAAGG | TGTCGGCAAA | GAAGCGAGAG | AATCTGTCTT |
| 1251 | GGCAGCGCTG | AAAGTCCTTG | AAGGTCTCGG | CGCTACATGG | GAAGAAGTGT |
| 1301 | CTCTTCCGCA | CAGTAAATAC | GCGCTTGCGA | CATATTACCT | GCTGTCATCT |

FIG. 3A

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| | | | | | |
|------|------------|-------------|------------|------------|-------------|
| 1351 | TCTGAAGCGT | CAGCGAACCT | TGCACGCTTT | GACGGCATCC | GCTACGGCTA |
| 1401 | CCGCACAGAC | AACGCGGATA | ACCTGATCGA | CCTTTACAAG | CAAACGCGCG |
| 1451 | CTGAAGGTTT | CGGAAATGAA | GTCAAACGCC | GCATCATGCT | CGGAACGTTT |
| 1501 | GCTTTAAGCT | CAGGCTACTA | CGATGCGTAC | TACAAAAAAG | CGCAAAAAGT |
| 1551 | GCGTACGTTG | ATTAAGAAGG | ATTTGAGGA | CGTATTTGAA | AAATATGATG |
| 1601 | TTATTGTTGG | ACCGACTACA | CCGACACCTG | CGTTTAAAAT | CGGTGAAAAC |
| 1651 | ACGAAGGATC | CGCTCACAAAT | GTACGCAAAC | GATATCTTAA | CGATTCCGGT |
| 1701 | CAACCTTGCG | GCGTACCGGG | AATCAGGTGC | CATGCGGTTA | GCAGACGGAC |
| 1751 | TTCCGCTCGG | CCTGCAAATC | ATCGGAAAAC | ACTTTGATGA | AGCACTGTAT |
| 1801 | ACCGCGTTGC | TCATGCATTT | GAACAAGCAA | CAGACCATCA | TAAAGCAAAA |
| | | B | START→ | | |
| 1851 | CCTGAACTGT | AAGGGGTGAA | AAGAATTGAA | CTTTGAAACG | GTAATCGGAC |
| 1901 | TTGAAGTCCA | CGTTGAGTTA | AAAACAAAAT | CAAAAATTTT | CTCAAGCTCT |
| 1951 | CCAACGCCAT | TCGGCGCGGA | GGCGAATACG | CAGACAAGCG | TTATTGACCT |
| 2001 | CGGATATCCG | GGCGTCCTGC | CTGTTCTGAA | CAAAGAAGCC | GTTGAATTCCG |
| 2051 | CAATGAAAGC | CGCTATGGCG | CTCAACTGTG | AGATCGCAAC | GGATACGAAG |
| 2101 | TTTGACCGCA | AAAACTATTT | CTATCCTGAC | AACCCGAAAG | CGTATCAGAT |
| 2151 | TTCTCAATTT | GATAAGCCAA | TCGGCGAAAA | CGGCTGGATC | GAAATTGAAG |
| 2201 | TCGGCGGCAA | AACAAAACGC | ATCGGCATCA | CGCGCCTTCA | TCTTGAAGAG |
| 2251 | GATGCCGGAA | AACTGACGCA | TACGGGCGAC | GGCTATTCTC | TTGTTGACTT |
| 2301 | CAACCGTCAA | GGAACGCCGC | TTGTTGAGTN | CGTATCAGAG | CCGGACATCC |
| 2351 | GCACGCCGGA | AGAANCGTAC | GCATATCTTG | AAAAGCTGAA | ATCCATCATC |
| 2401 | CAATATACAG | GCGTTTCTGA | CTGTAAAATG | GAAGAAGGCT | CACTTCGCTG |
| 2451 | TGACGCCAAT | ATCTCTCTTC | GTCCGATCGG | CCAAGAGGAA | TTCGGCACAA |
| 2501 | AAACAGAATT | GAAAACTTG | AACTCCTTTG | CGTTTGTTCA | AAAAGGCCTT |
| 2551 | GAGCATGAAG | AAAAACGCCA | GGAGCAGGTT | CTTCTTTCCG | GCTTCTTCAT |
| 2601 | CCAGCAAGAA | ACTCGCCGTT | ATGATGAAGC | AACGAAGAAA | ACCATTCTTA |
| 2651 | TGCGTGTCAA | AGAGGGATCT | GACGACTACC | GTTACTTCCC | AGAGCCAGAT |

FIG. 3B

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| | | | | | |
|------|------------|------------|------------|------------|--------------|
| 2701 | CTAGTCGAGC | TCTACATTGA | TGATGAATGG | AAGGAACGCG | TAAAAGCAAG |
| 2751 | CATTCCTGAG | CTTCCGGATG | AGCGCCGCAA | GCGTTATATC | GAAGAGCTTG |
| 2801 | GCTTCGCTGC | ATATGACGCA | ATGGTTCTGA | CGCTGACAAA | AGAAATGGCT |
| 2851 | GATTTCTTCG | AAGAAACCGT | TCAAAAAGGC | GCTGAAGCTA | AACAAGCGTC |
| 2901 | TAACTGGCTG | ATGGGTGAAG | TGTCAGCTTA | CCTAAACGCA | GAACAAAAAG |
| 2951 | AGCTTGCCGA | TGTTGCCCTG | ACACCTGAAG | GCCTTGCCGG | CATGATCAAA |
| 3001 | TTGATTGAAA | AAGGAACCAT | TTCTTCTAAG | ATCGCGAAGA | AAGTGTTTAA |
| 3051 | AGAATTGATT | GAAAAAGGCG | GCGACGCTGA | GAAGATTGTG | AAAGAGAAAAG |
| 3101 | GCCTTGTTCA | GATTTCTGAC | GAAGGCGTGC | TTCTGAAGCT | TGTCACTGAG |
| 3151 | GCGCTTGACA | ACAATCCTCA | ATCAATCGAA | GACTTTAAAA | ACGGAAAAGA |
| 3201 | CCGCGCGATC | GGCTTCCTAG | TCGGACAGAT | TATGAAAGCG | TCCAAAGGAC |
| 3251 | AAGCCAACCC | GCCGATGGTC | AACAAAATTC | TGCTTGAAGA | AATTAAAAAA |
| 3301 | CGCTAATAAA | AAAGCAGCCC | TTAGAGGCTG | CTTTTTTTAT | GGTCAAATTG |
| 3351 | AGATAAAGAC | AAGATGAGGG | CCCGAAGCCT | TTCAACTTCT | TTGTCGTTGG |
| 3401 | TTCCGGCCAA | ATTGGACAGC | ATGCCTTTAT | AATCGGCTTG | CGCGGTTTAT |
| 3451 | CCTGAGTCAA | TTCTTCCTCG | ATAAGATAAG | TGACACGGTG | <u>ATATC</u> |

3495
(EcoRV)

FIG. 3C

INTERNATIONAL SEARCH REPORT

Internat: Application No
PCT/US 98/01860

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/54 C12N9/10 C07K16/40 C12Q1/52

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| X | EP 0 191 221 A (UNIV CALIFORNIA) 20 August 1986 see page 16, line 22 - page 19, line 18 --- -/-- | 1 |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

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- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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Date of the actual completion of the international search

11 June 1998

Date of mailing of the international search report

02-07-1998

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Maddox, A

INTERNATIONAL SEARCH REPORT

Internat'l Application No
PCT/US 98/01860

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|
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Internat Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 14,15,16,22,23,66-70 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/01860

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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